```
=> d his

     (FILE 'HOME' ENTERED AT 09:28:23 ON 01 FEB 2002)

     FILE 'HCAPLUS' ENTERED AT 09:28:52 ON 01 FEB 2002
L1           47 S LPXA OR LPXB OR LPXD
L2        25280 S GRAM(W)NEGATIV?
L3            9 S L1 AND L2
L4         7104 S DIPLOCOCC? OR GONOCOCC? OR BORDETELL?
L5         2890 S (NEISSERIA OR N)(W)MENINGIT?
L6         3794 S (NEISSERIA OR N)(W)GONORRH?
L7         1426 S (BORDETALLA OR B)(W)PERTUSSIS
L8            5 S L3 AND L4-7
L9        61824 S VACCIN? OR IMMUNOSTIM?
L10           2 S L8 AND L9
L11          57 S VAN DER LEY P?/AU
L12          13 S STEEGHS L?/AU
L13           6 S L11-12 AND L2
L14           1 S L10 NOT L13
L15           1 S L8 NOT (L10 OR L13 OR L14)
L16           4 S L3 NOT (L10 OR L13-15)
L17           2 S L16 AND (OMP OR OUTER MEMBRANE)
L18           4 S L16 AND LIPID(W)A
L19           2 S L16 AND (LPS OR LIPOPOLYSACCHARID?)
L20           4 S L16-19

     FILE 'REGISTRY' ENTERED AT 09:42:46 ON 01 FEB 2002
               E LPXA/CN
L21           5 S E4-8
               E LPXB/CN
L22           4 S E4-7
               E LPXD/CN
L23           1 S E4
L24           1 S LPS/CN
               E LIPOPOLYSACCH/CN
L25          12 S E107-115
               E OUTER MEMBRANE/CN
L26        1429 S "OUTER MEMBRANE PROTEIN"
L27           1 S 90365-28-9

     FILE 'HCAPLUS' ENTERED AT 10:20:15 ON 01 FEB 2002

     FILE 'REGISTRY' ENTERED AT 10:22:41 ON 01 FEB 2002
L28           4 S L21 NOT PMS/CI
L29           3 S L22 NOT PMS/CI

     FILE 'HCAPLUS' ENTERED AT 10:24:13 ON 01 FEB 2002
L30           1 S L28-29 OR L23
L31           1 S L28 AND L30
L32          42 S L1 NOT (L10 OR L13-15 OR L31)
L33           4 S L2 AND L32
L34          27 S L32 AND ((LPS OR LIPOPOLYSACCHARID?) OR LIPID(W)A OR  (OMP OR
L35        4445 S L24-26
L36          24 S L27
L37           0 S L32 AND L35-36
L38          27 S L33 OR L34
L39           5 S L1 NOT L32
L40       35959 S L2 OR L4-7
L41        1260 S L40 AND ((DEFICIEN? OR LACK? OR NONE )(5A)(LPS OR LIPOPOLYSAC
L42           1 S L40 AND ((DEFICIEN? OR LACK? OR NONE )(5A)(LPS OR LIPOPOLYSAC
```

cites related to mutant genes

most specific cites; L1-20 focuses on named genes & bacteria

cites from inventor search

1 cite

1 cite

4 cites

looking for claimed genes, LPS, etc. in reg file

claim 8 cpd

getting rid of polymers

1 cite - only 1 cite related to specific sequences of genes

27 cites related to LPS or Lipid A or omp

5 remaining cites

remaining L1 cites

L43	0	S	L42 NOT (L10 OR L13-15 OR L31)
L44	102	S	L41 AND ((DEFICIEN? OR LACK? OR NONE)(5A)(LPS OR LIPOPOLYSAC
L45	12	S	L41 AND ((DEFICIEN? OR LACK? OR NONE)(5A)(LIPID(W)A))
L46	1159	S	L41 AND (OMP OR OUTER MEMBRANE PROTEIN)
L47	384	S	L44-46 AND L9
L48	12	S	L47 AND ((DEFICIEN? OR LACK? OR NONE OR FREE)(5A)(LPS OR LIPO
L49	10	S	L48 NOT (L10 OR L13-15 OR L31)
L50	23	S	L36 NOT (L10 OR L13-15 OR L31)

L49 → 10 cites (most general) related to L4-7 bacteria & Lipid A or Omp & free of LPS

L50 - 23 remaining cited cites related to cpd of claim 8

=> d ibib abs hitstr

L31 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:222685 HCAPLUS
DOCUMENT NUMBER: 134:232543
TITLE: Complete genomic sequence of Pasteurella multocida, Pm70
AUTHOR(S): May, Barbara J.; Zhang, Qing; Li, Ling Ling; Paustian, Michael L.; Whittam, Thomas S.; Kapur, Vivek
CORPORATE SOURCE: Department of Veterinary Pathobiology, University of Minnesota, St. Paul, MN, 55108, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (2001), 98(6), 3460-3465
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The complete genome sequence of a common avian clone of Pasteurella multocida, Pm70, is provided. The genome of Pm70 is a single circular chromosome 2,257,487 base pairs in length and contains 2014 predicted coding regions, 6 rRNA operons, and 57 tRNAs. Genome-scale evolutionary analyses based on pairwise comparisons of 1197 orthologous sequences between P. multocida, Haemophilus influenzae, and Escherichia coli suggest that P. multocida and H. influenzae diverged .apprxeq.270 million years ago and the .gamma. subdivision of the proteobacteria radiated about 680 million years ago. Two previously undescribed open reading frames, accounting for .apprxeq.1% of the genome, encode large proteins with homol. to the virulence-assocd. filamentous hemagglutinin of Bordetella pertussis. Consistent with the crit. role of iron in the survival of many microbial pathogens, in silico and whole-genome microarray analyses identified more than 50 Pm70 genes with a potential role in iron acquisition and metab. Overall, the complete genomic sequence and preliminary functional analyses provide a foundation for future research into the mechanisms of pathogenesis and host specificity of this important multispecies pathogen. The sequences of the genome and encoded proteins are available in the GenBank database under Accession No. AE004439.

IT **329812-30-8 329819-27-4 329830-36-6**
329830-37-7
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; complete genomic sequence of Pasteurella multocida)

RN 329812-30-8 HCAPLUS
CN LpxC (Pasteurella multocida strain IL1403 clone PM70 gene lpxC) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

RN 329819-27-4 HCAPLUS
CN LpxK (Pasteurella multocida strain IL1403 clone PM70 gene lpxK) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

RN 329830-36-6 HCAPLUS
CN Protein (Pasteurella multocida strain IL1403 clone PM70 gene lpxA) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

RN 329830-37-7 HCAPLUS
CN LpxB (Pasteurella multocida strain IL1403 clone PM70 gene lpxB) (9CI) (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 1

L49 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:473695 HCAPLUS
DN 135:209698
TI Dendritic cell activation and cytokine production induced by group B **Neisseria meningitidis**: interleukin-12 production depends on lipopolysaccharide expression in intact bacteria
AU Dixon, Garth L. J.; Newton, Phillippa J.; Chain, Benjamin M.; Katz, David; Andersen, Svein Rune; Wong, Simon; Van der Ley, Peter; Klein, Nigel; Callard, Robin E.
CS Immunobiology Unit, Institute of Child Health, London, WC1N 1EH, UK
SO Infect. Immun. (2001), 69(7), 4351-4357
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB Interactions between dendritic cells (DCs) and microbial pathogens are fundamental to the generation of innate and adaptive immune responses. Upon stimulation with bacteria or bacterial components such as lipopolysaccharide (LPS), immature DCs undergo a maturation process that involves expression of costimulatory mols., HLA mols., and cytokines and chemokines, thus providing crit. signals for lymphocyte development and differentiation. In this study, we investigated the response of in vitro-generated human DCs to a serogroup B strain of **Neisseria meningitidis** compared to an isogenic mutant lpxA strain totally **deficient** in **LPS** and purified **LPS** from the same strain. We show that the parent strain, lpxA mutant, and meningococcal LPS all induce DC maturation as measured by increased surface expression of costimulatory mols. and HLA class I and II mols. Both the parent and lpxA strains induced prodn. of tumor necrosis factor alpha (TNF-.alpha.), interleukin-1.alpha. (IL-1.alpha.), and IL-6 in DCs, although the parent was the more potent stimulus. In contrast, high-level IL-12 prodn. was only seen with the parent strain. Compared to intact bacteria, purified LPS was a very poor inducer of IL-1.alpha., IL-6, and TNF-.alpha. prodn. and induced no detectable IL-12. Addn. of exogenous LPS to the lpxA strain only partially restored cytokine prodn. and did not restore IL-12 prodn. These data show that non-LPS components of **N. meningitidis** induce DC maturation, but that LPS in the context of the intact bacterium is required for high-level cytokine prodn., esp. that of IL-12. These findings may be useful in assessing components of **N. meningitidis** as potential **vaccine** candidates.
RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 2

L49 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:651452 HCAPLUS
DN 131:335495
TI Immunogenicity of **outer membrane proteins** in a **lipopolysaccharide-deficient** mutant of **Neisseria meningitidis**: influence of adjuvants on the immune response
AU Steeghs, Liana; Kuipers, Betsy; Hamstra, Hendrik Jan; Kersten, Gideon; Van Alphen, Loek; Van der Ley, Peter
CS Laboratory of Vaccine Research, National Institute of Public Health and the Environment, Bilthoven, 3720 BA, Neth.
SO Infect. Immun. (1999), 67(10), 4988-4993
CODEN: INFIBR; ISSN: 0019-9567
PB American Society for Microbiology
DT Journal
LA English
AB The immunogenicity of outer membrane complexes (OMCs) or heat-inactivated bacteria of a **lipopolysaccharide** (**LPS**)-**deficient** mutant derived from meningococcal strain H44/76 was studied. The immune response in BALB/c mice to the major **outer membrane proteins** was poor compared to the immune response elicited by wild-type immunogens. However, addn. of external H44/76 LPS to mutant OMCs entirely restored the immune response. By using an **LPS-deficient** mutant, it may be possible to substitute a less toxic compd. as adjuvant in meningococcal outer membrane **vaccines**. Therefore, a broad panel of adjuvants were tested for their potential to enhance the immunogenicity of **LPS-deficient** OMCs. AlPO4, Rhodobacter sphaeroides LPS, monophosphoryl lipid A and alkali-hydrolyzed meningococcal LPS showed significantly lower adjuvant activity than did H44/76 LPS. Adjuvant activity similar to H44/76 LPS was found for Escherichia coli LPS, meningococcal icsB and rfaC LPS, QuilA, subfractions of QuilA, and MF59. Good adjuvant activity was also found with meningococcal htrB1 LPS, contg. penta-acylated lipid A. Antisera elicited with the less active adjuvants showed relatively high IgG1 titers, whereas strong adjuvants also induced high IgG2a and IgG2b responses in addn. to IgG1. Antisera with the IgG2a and IgG2b isotypes showed high bactericidal activity, indicating that adjuvants promoting the IgG2a and IgG2b response contribute most to the protective mechanism. Thus, this study demonstrates that the immunogenicity of meningococcal **LPS-deficient** OMCs can be restored by using less toxic adjuvants, which opens up new avenues for development of **vaccines** against meningococcal disease.
RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 3

L49 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1998:761797 HCAPLUS
DN 130:17217
TI **Vaccine** against lipopolysaccharide core
IN Bennett-Guerrero, Elliott; Barclay, George Robin; Poxton, Ian Raymond; McIntosh, Thomas James; Snyder, David Scott
PA Medical Defense Technologies, Llc, USA
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9851217	A1	19981119	WO 1998-US9988	19980515
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9874912	A1	19981208	AU 1998-74912	19980515
	EP 1011440	A1	20000628	EP 1998-922339	19980515
	R: BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE				
	JP 2001527562	T2	20011225	JP 1998-549589	19980515
PRAI	US 1997-46680	P	19970516		
	WO 1998-US9988	W	19980515		

AB Complete core **LPS** (**lacking** O-polysaccharide side chains) from **Gram-neg**. bacteria are incorporated into a **vaccine** typically in liposomes. The complete core of E. coli K 12 is particularly useful. Upon administration to a mammal the **vaccine** stimulates synthesis of antibodies which are cross-protective against smooth and rough forms of LPS from at least two different **Gram-neg**. bacterial strains having different core structures.

RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d bib abs 4

L49 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1998:192150 HCAPLUS
DN 128:252977
TI Lipopolysaccharide-binding protein derivatives for treatment of **gram-negative** bacterial infection
IN Gazzano-Santoro, Helene; Theofan, Georgia; Trown, Patrick W.
PA Xoma Corp., USA
SO U.S., 67 pp. Cont.-in-part of U.S. Ser. No. 79,510, abandoned.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5731415	A	19980324	US 1994-261660	19940617
PRAI	US 1993-79510		19930617		

AB Disclosed are novel biol. active lipopolysaccharide binding protein (LBP) derivs. including LBP deriv. hybrid proteins which are characterized by the ability to bind to and neutralize **LPS** and which **lack** the CD14-mediated **immunostimulatory** properties of holo-LBP.

=> d bib abs 5

L49 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1995:492022 HCAPLUS
DN 122:232671
TI Lipopolysaccharide binding protein derivatives, their manufacture with recombinant cells, and their use in treatment of **Gram-neg**. bacterial infections
IN Gazzano-Santoro, Helene; Theofan, Georgia; Trown, Patrick W.
PA Xoma Corp., USA
SO PCT Int. Appl., 108 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9500641	A1	19950105	WO 1994-US6931	19940617
	W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9471756	A1	19950117	AU 1994-71756	19940617
PRAI	US 1993-79510		19930617		
	WO 1994-US6931		19940617		

AB Disclosed are novel biol. active lipopolysaccharide binding protein (LBP) derivs. including LBP deriv. hybrid proteins which are characterized by the ability to bind to and neutralize **LPS** and which **lack** the CD14-mediated **immunostimulatory** properties of holo-LBP. CDNA's for human LBP and for (1-197)LBP, called LBP25 were cloned. Genes for LBP25, for BPI23 [where BPI refers to human bactericidal/permeability-increasing protein and BPI23 to (1-199)BPI], and hybrid LBP-BPI proteins were constructed and expressed in CHO cells. Lipid A binding activity and pharmacokinetics of selected proteins were examd. LBP25, unlike LBP, did not potentiate release of tumor necrosis factor by peripheral blood mononuclear cells and did not mediate LPS-stimulated tissue factor prodn. LBP25 completely inhibited LPS induction of endothelial cell adhesiveness for neutrophils. Addnl., LBP25 was unable to mediate CD14-dependent enhanced binding of bacteria to monocytes.

=> d bib abs 6

L49 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1993:515323 HCAPLUS
DN 119:115323
TI Conjugates of the class II protein of the outer membrane of **Neisseria meningitidis** and of human immunodeficiency virus 1 (HIV-1)-related peptides
IN Emini, A.; Liu, Margaret A.; Marburg, Stephen; Tolman, Richard L.
PA Merck and Co., Inc., USA
SO Eur. Pat. Appl., 66 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 519554	A1	19921223	EP 1992-201693	19920611
R: CH, DE, FR, GB, IT, LI, NL				
CA 2071088	AA	19921220	CA 1992-2071088	19920611
JP 05306299	A2	19931119	JP 1992-201740	19920619
PRAI US 1991-715273		19910619		

AB The class II major immunoenhancing protein (MIEP) of **N. meningitidis** (purified directly from the outer membrane of **N. meningitidis** or obtained through recombinant cloning and expression of DNA encoding the **N. meningitidis** MIEP) has immunol. carrier as well as immunol. enhancement and mitogenic properties. MIEP conjugates with HIV-1-related peptides are useful for the induction of mammalian immune responses directed against the peptides, against HIV-1 strains, and for the neutralization of HIV-1 and prevention of HIV-1-related diseases. Synthesis of HIV PND (principal neutralizing determinant) peptides is described, as is conjugation of these peptides to MIEP. Monkeys inoculated with 2 such conjugates developed antibodies specifically capable of binding the resp. PND peptide. Unconjugated, disulfide-bonded, cyclic peptide having identical primary sequence did not raise detectable anti-peptide antibodies in monkeys at 0, 4, or 8 wks. MIEP, **free** of detectable **lipopolysaccharide**, showed mitogenic activity (lymphocyte proliferation).

=> d bib abs 7

L49 ANSWER 7 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1991:469825 HCAPLUS
DN 115:69825
TI Cross-protective Salmonella **vaccines** using multiply mutant S. typhimurium
IN Curtiss, Roy, III; Munson, Maryann
PA Washington University, USA
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9106317	A1	19910516	WO 1990-US6503	19901102
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
	CA 2072633	AA	19910504	CA 1990-2072633	19901102
	AU 9067371	A1	19910531	AU 1990-67371	19901102
	EP 500699	A1	19920902	EP 1990-917076	19901102
	EP 500699	B1	19980610		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE				
	JP 05504331	T2	19930708	JP 1990-515888	19901102
	AT 167061	E	19980615	AT 1990-917076	19901102
PRAI	US 1989-431597		19891103		
	WO 1990-US6503		19901102		

AB Attenuated Salmonella for use as live **vaccines** against Salmonella and other **Gram-neg.** bacteria are prepd. The organisms are incapable of manufg. the lipopolysaccharide involved in pathogenesis because of mutation in several genes involved in the synthesis of, or regulation of synthesis of, the lipopolysaccharide. Other genes involved in the regulation of pathogenesis-related genes are also inactivated. A S. typhimurium with the crp and cya genes deleted was prepd. by transposon mutagenesis with Tn10. S. typhimurium carrying both deletions had an LD50 of >109 colony-forming units (CFU) in Leghorn chicks, vs. 2 .times. 104 - 2 .times. 105 for wild-types. Similar deletions of the phoP, fur, pmi, and galE genes were constructed. Some of the constructs prepd. were found to confer cross-resistance to S. enteriditis and pathogenic Escherichia coli.

=> d bib abs 8

L49 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1989:580823 HCAPLUS
DN 111:180823
TI Measurements of lipopolysaccharide (endotoxin) in meningococcal protein and polysaccharide preparations for **vaccine** usage
AU Tsai, C. M.; Frasch, C. E.; Rivera, E.; Hochstein, H. D.
CS Cent. Biol. Eval. Res., FDA, Bethesda, MD, 20892, USA
SO J. Biol. Stand. (1989), 17(3), 249-58
CODEN: JBSTBI; ISSN: 0092-1157
DT Journal
LA English
AB Lipopolysaccharide (LPS, i.e. endotoxin) present in meningococcal **outer-membrane protein** and polysaccharide preprns. made for **vaccine** use was quantitated by a silver-stain method following SDS-PAGe. The reactivities of LPS in the preprns. were also measured by rabbit pyrogenicity and Limulus amebocyte lysate (LAL) assay. Although rabbit pyrogenicity and LAL assay are more sensitive than the silver stain method, the latter provided an actual amt. of LPS present in the protein or in the polysaccharide. For a meningococcal protein prepn., rabbit pyrogenicity showed about 1/10, and even less by LAL assay, of the actual amt. of LPS. This is because protein-bound LPS in meningococcal protein preprns. is about 10-fold less active in causing fever in rabbits, and 20- to 40-fold less active in the gelation of LAL than the same amout of a purified **free LPS** which is generally used as a ref. in quantitating LPS in these two assays. As for the small amt. of LPS present in a meningococcal polysaccharide prepn., similar LPS content was obtained when measured by the 3 methods suggesting that the LPS is not bound to the polysaccharide in contrast to that in the proteins mentioned above. The purified meningococcal LPS was pyrogenic in rabbits at 1 ng/kg.

=> d bib abs 9

L49 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1986:558791 HCAPLUS
DN 105:158791
TI Detoxified polysaccharide-**outer membrane protein** complexes and their use as antibacterial **vaccines**
IN Zollinger, Wendell D.; Boslego, John W.; Moran, Elizabeth Ellen; Brandt, Brenda; Collins, Hugh H.; Mandrell, Robert E.; Altieri, Patricia; Berman, Sanford
PA United States Dept. of the Army, USA
SO U. S. Pat. Appl., 51 pp.
CODEN: XAXXAV
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 777068	A0	19860328	US 1985-777068	19850917
US 4707543	A	19871117		

AB **Outer membrane proteins** of **gram-neg.** bacteria are prepd. relatively **free** from toxic **lipopolysaccharide** (<1%) and solubilized with a mixt. of polysaccharides for use in a **vaccine.** For example, the outer membrane complex from **Neisseria meningitidis** serotype 2b was dissolved in Tris-EDTA-Empigen BB buffer (pH 8.0) with sonication and sepd. from lipopolysaccharide by repeated (NH4)2SO4 pptn. The purified **outer membrane proteins** were sterilized by filtration, mixed with a sterile capsular polysaccharide mixt. from serogroups A, C, Y, and W-135, and the complex was pptd. with EtOH and suspended in 3% lactose soln. to provide an injectable soln. The antibody response to this **vaccine** in mice was greater than that to the capsular polysaccharides alone.

=> d bib abs 10

L49 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2002 ACS
AN 1984:460118 HCAPLUS
DN 101:60118
TI Antigen compositions containing **Neisseria meningitidis** polysaccharide-protein complexes for **vaccines**
IN Moreno, Carlos; Lifely, Mark Robert
PA Wellcome Foundation Ltd., UK
SO Eur. Pat. Appl., 30 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 109688	A2	19840530	EP 1983-111621	19831121
	EP 109688	A3	19861203		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	JP 59176214	A2	19841005	JP 1983-220497	19831122
	US 4753796	A	19880628	US 1986-883470	19860711
PRAI	GB 1982-33317		19821123		
	GB 1983-16950		19830622		
	GB 1983-16951		19830622		
	US 1983-554055		19831121		

AB Antigenic bacterial capsular polysaccharide-**outer membrane protein** complexes (**free** of cells and **lipopolysaccharides**) were isolated from **Neisseria meningitidis**. **N. meningitidis** Cultures were mixed with a quaternary ammonium salt. The resulting ppt. was mixed with a water sol. Ca or Mg salt and an aq. medium to form a soln. A lower alkanol was then added to ppt. the complex. Further, the antigenic prepn. was complexed with a metal (Al, Ca, Fe, Ni, or Zn) to increase its immunogenicity. Such polysaccharide-protein complexes were immunogenic and protected mice against mortality due to **N. meningitidis** infections. These **vaccines** can be made to protect against serogroup B, serotype 6 or serogroup B, serotype 2 **N. meningitidis**.

=> d ibib abs hitstr 1

L50 ANSWER 1 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:819945 HCAPLUS
DOCUMENT NUMBER: 134:143735
TITLE: Comparative functional characterization in vitro of heptosyltransferase I (WaaC) and II (WaaF) from Escherichia coli
AUTHOR(S): Gronow, Sabine; Brabetz, Werner; Brade, Helmut
CORPORATE SOURCE: Division of Medical and Biochemical Microbiology, Research Center Borstel, Center for Medicine and Biosciences, Borstel, D-23845, Germany
SOURCE: Eur. J. Biochem. (2000), 267(22), 6602-6611
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

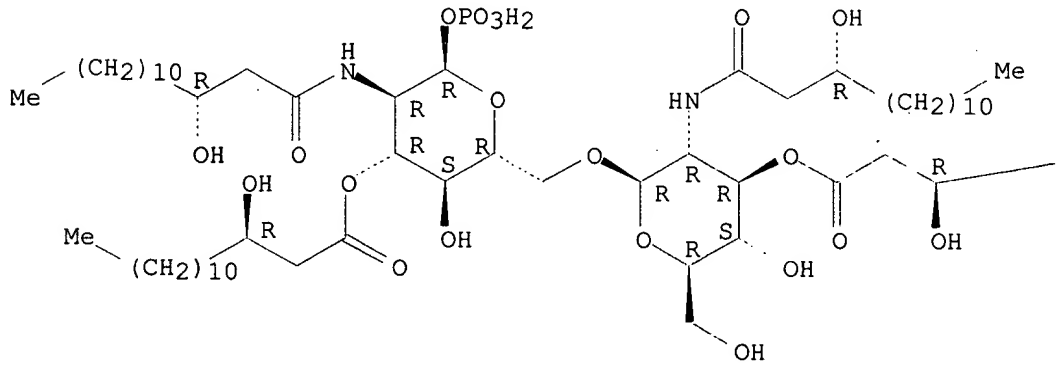
AB Heptosyltransferase II, encoded by the waaF gene of Escherichia coli, is a glycosyltransferase involved in the synthesis of the inner core region of lipopolysaccharide. The gene was subcloned from plasmid pWSB33 into a shuttle vector for the expression in the gram-pos. host Corynebacterium glutamicum. The in vitro activity of the enzyme was investigated in comparison to that of heptosyltransferase I (WaaC) using as a source for the sugar nucleotide donor, ADP-L-glycero-D-manno-heptose, a low mol. mass filtrate from a .DELTA.waaCF E. coli strain. Synthetic lipid A analogs varying in the acylation or phosphorylation pattern or both were tested as acceptors for the subsequent transfer of 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) and heptose by successive action of Kdo transferase (WaaA), heptosyltransferase I (WaaC) and heptosyltransferase II (WaaF). The reaction products were characterized after sepn. by TLC and blotting with monoclonal antibodies specific for the acceptor, the intermediates and the final products.

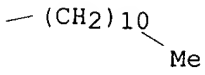
IT **90365-28-9**, Compound 405
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(comparative functional characterization in vitro of heptosyltransferase I (WaaC) and II (WaaF) from Escherichia coli)

RN 90365-28-9 HCAPLUS
CN .alpha.-D-Glucopyranose, 2-deoxy-6-O-[2-deoxy-3-O-[(3R)-3-hydroxy-1-oxotetradecyl]-2-[[(3R)-3-hydroxy-1-oxotetradecyl]amino]-.beta.-D-glucopyranosyl]-2-[[(3R)-3-hydroxy-1-oxotetradecyl]amino]-, 1-(dihydrogen phosphate) 3-[(3R)-3-hydroxytetradecanoate] (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A





REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs 2-23

L50 ANSWER 2 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:233827 HCAPLUS
DOCUMENT NUMBER: 130:264044
TITLE: Escherichia coli lipid A 4'-kinase and its recombinant production and use for synthesis of lipid A analogs
INVENTOR(S): Raetz, Christian R. H.; Garrett, Teresa A.; Kadrmas, Julie L.
PATENT ASSIGNEE(S): Duke University, USA
SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9916473	A1	19990408	WO 1998-US10097	19980518
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1011731	A1	20000628	EP 1998-923475	19980518
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
AU 9875764	A1	19990423	AU 1998-75764	19980624

PRIORITY APPLN. INFO.: US 1997-46947 P 19970519
WO 1998-US10097 W 19980518

AB The present invention relates, in general, to lipid A 4'-kinase and, in particular, to a nucleic acid encoding lipid A 4'-kinase and to a method of producing lipid A 4' kinase recombinantly using same. The predicted amino acid sequence of the orfE(lpxK) gene product of Escherichia coli comprises 328 amino acids in length and catalyzes the phosphorylation of lipid A precursors at the 4' position. The invention further relates to methods of producing 4' phosphorylated lipid A analogs using the recombinantly produced lipid A 4'-kinase.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 3 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:330379 HCAPLUS
DOCUMENT NUMBER: 129:77221
TITLE: Accumulation of a lipid A precursor lacking the 4'-phosphate following inactivation of the Escherichia coli lpxK gene
AUTHOR(S): Garrett, Teresa A.; Que, Nanette L. S.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1998), 273(20), 12457-12465
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lpxK gene has been proposed to encode the lipid A 4'-kinase in Escherichia coli (Garrett, T. A., Kadrmas, J. L., and Raetz, C. R. H. (1997) J. Biol. Chem. 272, 21855-21864). In cell exts., the kinase

phosphorylates the 4'-position of a tetraacyldisaccharide 1-phosphate precursor (DS-1-P) of lipid A, but the enzyme has not yet been purified because of instability. Gene lpxK is co-transcribed with an essential upstream gene, msbA, with strong homol. to mammalian Mdr proteins and ABC transporters. Gene msbA may be involved in the transport of newly made lipid A from the inner surface of the inner membrane to the outer membrane. Insertion of an .OMEGA.-chloramphenicol cassette into msbA also halts transcription of lpxK. The authors have now constructed a strain in which only the lpxK gene is inactivated by inserting a kanamycin cassette into the chromosomal copy of lpxK. This mutation is complemented at 30.degree. by a hybrid plasmid with a temp.-sensitive origin of replication carrying lpxK+. When this strain (designated TG1/pTAG1) is grown at 44.degree., the plasmid bearing the lpxK+ is lost, and the phenotype of an lpxK knock-out mutation is unmasked. The growth of TG1/pTAG1 was inhibited after several hours at 44.degree., consistent with lpxK being an essential gene. Furthermore, 4'-kinase activity in exts. made from these cells was barely detectable. In accordance with the proposed biosynthetic pathway for lipid A, DS-1-P (the 4'-kinase substrate) accumulated in TG1/pTAG1 cells grown at 44.degree.. The DS-1-P from TG1/pTAG1 was isolated, and its structure was verified by 1H NMR spectroscopy. DS-1-P had not been isolated previously from bacterial cells. Its accumulation in TG1/pTAG1 provides addnl. support for the pathway of lipid A biosynthesis in E. coli. Homologs of lpxK are present in the genomes of other Gram-neg. bacteria.

L50 ANSWER 4 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:577492 HCAPLUS
DOCUMENT NUMBER: 127:273586
TITLE: Identification of the gene encoding the Escherichia coli lipid A 4'-kinase. Facile phosphorylation of endotoxin analogs with recombinant LpxK
AUTHOR(S): Garrett, Teresa A.; Kadrmas, Julie L.; Raetz, Christian R. H.
CORPORATE SOURCE: Dep. Biochem., Duke Univ. Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1997), 272(35), 21855-21864
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The genes for 7 of 9 enzymes needed for the biosynthesis of Kdo2-lipid A (Re endotoxin) in Escherichia coli have been reported. The present study identifies a novel gene encoding the lipid A 4'-kinase (the 6th step of the pathway). The 4'-kinase transfers the .gamma.-phosphate of ATP to the 4'-position of a tetraacyldisaccharide 1-phosphate intermediate (termed DS-1-P) to form tetraacyldisaccharide 1,4'-bis-phosphate (lipid IVA). The 4'-phosphate is required for the action of distal enzymes, such as Kdo transferase and also renders lipid A substructures active as endotoxin antagonists or mimetics. Lysates of E. coli generated using individual .lambda. clones from the ordered Kohara library were assayed for overprodn. of 4'-kinase. Only one clone, [218]E1D1, which directed 2-2.5-fold overprodn., was identified. This construct contains 20 kbp of E. coli DNA from the vicinity of minute 21. Two genes related to the lipid A system map in this region: msbA, encoding a putative translocator, and kdsB, the structural gene for CMP-Kdo synthase. MsbA forms an operon with a downstream, essential open reading frame of unknown function, designated orfE. OrfE was cloned into a T7 expression system. Washed membranes from cells overexpressing orfE display .apprx.2000-fold higher specific activity of 4'-kinase than membranes from cells with vector

alone. Membranes contg. recombinant, overexpressed 4'-kinase (but not membranes with wild-type kinase levels) efficiently phosphorylate three DS-1-P analogs: 3-aza-DS-1-P, base-treated DS-1-P, and base-treated 3-aza-DS-1-P. A synthetic hexaacylated DS-1-P analog, compd. 505, can also be phosphorylated by membranes from the overproducer, yielding [4'-32P]lipid A (endotoxin). The overexpressed lipid A 4'-kinase is very useful for making new 4'-phosphorylated lipid A analogs with potential utility as endotoxin mimetics or antagonists. OrfE is suggested to be the structural gene for the 4'-kinase and is redesignated lpxK.

L50 ANSWER 5 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:321297 HCAPLUS
DOCUMENT NUMBER: 120:321297
TITLE: The significance of the hydrophilic backbone and the hydrophobic fatty acid regions of lipid A for macrophage binding and cytokine induction
AUTHOR(S): Kirikae, Teruo; Schade, F. Ulrich; Zahringer, Ulrich; Kirikae, Fumiko; Brade, Helmut; Kusumoto, Shoichi; Kusama, Tsuneo; Rietschel, Ernst Th.
CORPORATE SOURCE: Inst. fur Exp. Biol. und Med., Forschungsinst. Borstel, Borstel, D-23845, Germany
SOURCE: FEMS Immunol. Med. Microbiol. (1994), 8(1), 13-26
CODEN: FIMIEV; ISSN: 0928-8244
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Natural partial structures of lipopolysaccharide (LPS) as well as synthetic analogs and derivs. of lipid A were compared with respect to inhibit the binding of 125I-labeled Re-chemotype LPS to mouse macrophage-like J774.1 cells and to induce cytokine-release in J774.1 cells. LPS, synthetic Escherichia coli-type lipid A (compd. 506) and tetraacyl precursor Ia (compd. 406) inhibited the binding of 125I-LPS to macrophage-like J774.1 cells and induced the release of tumor necrosis factor .alpha. (TNF.alpha.) and interleukin 6 (IL-6). Deacylated R-chemotype LPS prepns. were completely inactive in inhibiting binding and in inducing cytokine-release. Among tetraacyl compds., the inhibition-capacity of LPS-binding was in decreasing order: PE-4 (.alpha.-phosphonooxyethyl analog of 406) > 406 .mchgt. 404 (4'-monophosphoryl partial structure of 406) > 405 (1-monophosphoryl partial structure of 406). In the case of hexaacyl prepns., compds. 506, PE-1 (.alpha.-phosphonooxyethyl analog of 506), and PE-2 (differing from PE-1 in having 14:0 at positions 2 and 3 of the reducing GlcN) inhibited LPS-binding and induced cytokine release equally well, whereas prepn. PE-3 (differing from PE-2 in contg. a .beta.-phosphonooxyethyl group) showed a substantially lower capacity in binding inhibition and cytokine induction. The conclusion is that chem. changes in the hydrophilic lipid A backbone reduce the capacity of lipid A to bind to cells, whereas the no. of fatty acids dets. the capacity of lipid A to activate cells. Thus, the bisphosphorylated hexosamine backbone of lipid A is essential for specific binding of LPS to macrophages and the acylation pattern plays a crit. role for LPS-promoted cell activation, i.e. cytokine induction.

L50 ANSWER 6 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:241944 HCAPLUS
DOCUMENT NUMBER: 120:241944
TITLE: The antibody reactivity of monoclonal lipid A antibodies is influenced by the acylation pattern of lipid A and the assay system employed
AUTHOR(S): Kuhn, Hella Monika; Brade, Lore; Appelmelk, Ben J.; Kusumoto, Shoichi; Rietschel, Ernst T.; Brade, Helmut
CORPORATE SOURCE: Div. Biochem. Microbiol., Inst. Exp. Biol. Med.,

Borstel, Germany
SOURCE: Immunobiology (Stuttgart) (1993), 189(5), 457-71
CODEN: IMMND4; ISSN: 0171-2985
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The influence of the acylation pattern of lipid A on the reactivity of murine monoclonal antibodies (mAb) was tested in different assay systems with synthetic lipid A antigens. Both the no. and type of fatty acids had an impact on the antigen amts. needed for optimal sensitization of sheep red blood cells, on the inhibition capacity of compds., and on the reactive antigen amts. in enzyme immunoassay and dot blot assay. Results obtained with two pentaacyl isomers indicated that the location of fatty acids is of no importance. Although all mAbs used recognized epitopes residing in the hydrophilic backbone of lipid A, their reactivities were greatly influenced by the no. as well as the type of acyl chains present. In the various assays, the mAbs reacted either similarly or discrepantly suggesting that epitopes are exposed differently in the test systems. Thus, for the detn. of the reactivity of lipid A mAbs it is useful and sometimes necessary to run various assays in parallel and to compare mAbs on the basis of reaction patterns.

L50 ANSWER 7 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:161151 HCAPLUS
DOCUMENT NUMBER: 120:161151
TITLE: Cross-binding activity and protective capacity of monoclonal antibodies to lipid A
AUTHOR(S): Mitov, Ivan; Freudenberg, Marine; Bamberger, Uwe; Galanos, Chris
CORPORATE SOURCE: Max-Planck-Inst. Immunbiol., Freiburg, Germany
SOURCE: Immunobiology (Stuttgart) (1993), 188(1-2), 1-12
CODEN: IMMND4; ISSN: 0171-2985
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Six hybridoma clones (1M, 4M, 9M, 11M, 18M, and 31G), secreting monoclonal antibodies (mAbs) against lipid A were obtained after fusion between cells of mouse myeloma line X63-Ag8.653 and spleen cells from BALB/c mice immunized with acid treated Salmonella minnesota bacteria coated with addnl. free lipid A. The specificity and cross-binding activity of the mAbs were characterized in ELISA by using synthetic lipid A analogs as well as different lipid A and lipopolysaccharides (LPS) extd. from R- and S-form bacteria. It was found that the antibodies recognize epitopes in which phosphate groups, esp. those at the C4' position of the glucosamine backbone of lipid A, were present. These epitopes were accessible also for the antibodies in purified intact LPS. By using a set of core glycolipids with increasing completion of the core region of the mol. and S-LPS it was shown that the mAbs cross-reacted with a variety of R- and S-form LPS. The binding activity decreased with increasing length of the polysaccharide chain. The mAb did not prevent ultimate lethality of mice challenged with Klebsiella pneumoniae B and S. typhimurium C5. However a delay of mortality rate of mice pretreated with antibodies 18M and 31G and infected with K. pneumoniae was seen.

L50 ANSWER 8 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:166912 HCAPLUS
DOCUMENT NUMBER: 118:166912
TITLE: Immune response of rabbits to lipid A: Influence of immunogen preparation and distribution of various lipid A specificities
AUTHOR(S): Kuhn, Hella Monika
CORPORATE SOURCE: Forschungsinst. Borstel, Inst. Exp. Biol. Med.,

Borstel, D-2061, Germany
SOURCE: Infect. Immun. (1993), 61(2), 680-8
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sixty-two rabbit anti-lipid A serum samples were compared with respect to the immunogens used (synthetic lipid A and partial structures, natural lipid A, or acid-treated bacteria). Ig type-specific differences in rabbit response between liposomal membrane-embedded (LME) and other lipid A immunogens were found: LME lipid A elicited predominantly IgM antibodies. Previous findings of equally good immune responses to synthetic lipid A and acid-treated bacterial (L. Brade, et. al., 1987) turned out to be restricted to complement-fixing antibodies; IgG titers of sera against free lipid A (whether synthetic or natural) were significantly lower than those raised with bacteria. The results indicated an increase in IgG content of sera from LME lipid A over other free lipid A immunogens to acid-treated bacteria. These data underline the importance of the physicochem. environment for the immunogenicity of lipid A. As a second objective, the presence of various lipid A antibody specificities was tested with synthetic lipid A antigens. Antibodies to monophosphoryl lipid A were detected only in sera raised with monophosphoryl immunogens. Reactivity with monosaccharide partial structures of lipid A was found both in sera against monophosphoryl lipid A and in 60% of sera against bisphosphoryl lipid A. In the former, monosaccharide reactivity was of a magnitude similar to that of reactivity with lipid A; in sera against bisphosphoryl lipid A, it was lower. No reactivity or only marginal reactivity was found with phosphate-free lipid A, thus emphasizing the role of phosphate substitution for the lipid A epitopes recognized.

L50 ANSWER 9 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:529548 HCAPLUS
DOCUMENT NUMBER: 117:129548
TITLE: Characterization of the epitope specificity of murine monoclonal antibodies directed against lipid A
AUTHOR(S): Kuhn, Hella Monika; Brade, Lore; Appelmelk, Ben J.; Kusumoto, Shoichi; Rietschel, Ernst T.; Brade, Helmut
CORPORATE SOURCE: Div. Biochem. Microbiol., Inst. Exp. Biol. Med., Borstel, D-2061, Germany
SOURCE: Infect. Immun. (1992), 60(6), 2201-10
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A series of monoclonal antibodies directed against lipid A was characterized by using synthetic lipid A analogs and partial structures. These compds. vary in phosphate substitution, acylation pattern (type, no., and distribution of fatty acids), and, in the case of monosaccharides, in their backbone glycosyl residue. The monoclonal antibodies tested could be subdivided into 5 groups according to their reactivity patterns. One group reacted exclusively with 1,4'-bisphosphoryl lipid A, and a 2nd also reacted with 4'-monophosphoryl lipid A. Two further groups recognized either 4-phosphoryl or 1-phosphoryl monosaccharide partial structures of lipid A. The 5th group reacted with 4-phosphoryl monosaccharide structures and with phosphate-free compds. Antibodies reactive with monosaccharide structures also recognized their epitopes in corresponding phosphorylated disaccharide compds. Both groups of monosaccharide and monophosphoryl lipid A-recognizing antibodies have access to their epitopes in bisphosphoryl compds. as well. Because of this unidirectional reactivity with more complex structures, the various specificities cannot be

distinguished by using bisphosphoryl lipid A (e.g., Escherichia coli lipid A) as a test antigen. The epitopes recognized by the various monoclonal antibodies all reside in the hydrophilic backbone of lipid A, and there was no indication that fatty acids were part of the epitopes recognized. Nevertheless, the reactivities of compds. in the different test systems are strongly influenced by their acylation patterns; i.e., acyl groups may modulate the exposure of lipid A epitopes.

L50 ANSWER 10 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:255932 HCAPLUS
DOCUMENT NUMBER: 116:255932
TITLE: Enzymic synthesis and comparative biological evaluation of a phosphonate analog of the lipid A precursor
AUTHOR(S): Scholz, Dieter; Bednarik, Karl; Ehn, Gerald; Neruda, Wolfgang; Janzek, Evelyne; Loibner, Hans; Briner, Karin; Vasella, Andrea
CORPORATE SOURCE: Sandoz Forschungsinst., Vienna, Austria
SOURCE: J. Med. Chem. (1992), 35(11), 2070-4
CODEN: JMCMAR; ISSN: 0022-2623
DOCUMENT TYPE: Journal
LANGUAGE: English
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* STRUCTURE DIAGRAM TOO LARGE FOR DISPLAY - AVAILABLE VIA OFFLINE PRINT *

AB Phosphonate analog I [R = (CH2)10Me, R1 = PO3H2] (II) of the lipid A precursor I (R1 = OPO3H2) (III) has been prepd. from nucleotide IV and phosphonate V in presense of lipid A synthase isolated from E. coli mutant 1061 or JB1104. The biol. properties of II and III are quite similar to each other as compared in the limulus amoebocyte lysate assay, by the activation of the RAW264 murine macrophagelike cell line (detd. by stimulation of ornithine decarboxylase), and by the pyrogenicity in rabbits. Hydrolytic removal of the 1-phosphate group of III is thus not prerequisite for its biol. activity.

L50 ANSWER 11 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:536543 HCAPLUS
DOCUMENT NUMBER: 115:136543
TITLE: C-Glycosidic analogs of lipid A and lipid X: synthesis and biological activities
AUTHOR(S): Vyplel, Hermann; Scholz, Dieter; Macher, Ingolf; Schindlmaier, Karl; Schuetze, Eberhard
CORPORATE SOURCE: Sandoz Forschungsinst., Vienna, A-1235, Austria
SOURCE: J. Med. Chem. (1991), 34(9), 2759-67
CODEN: JMCMAR; ISSN: 0022-2623
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 115:136543
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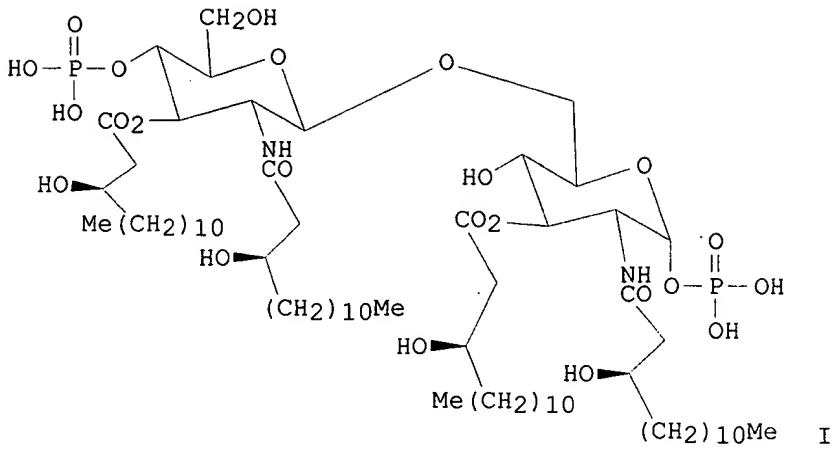

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AB The synthesis of a series of novel analogs of lipid A (I), the lipophilic terminal of lipopolysaccharides (LPS), and lipid X, the reducing monosaccharide unit in lipid A, is reported. In these compds., the native 1-O-phosphate group was replaced by a "bioisosteric" CH2COOH substituent. The new N,O-acylated monosaccharide C-glycosides were obtained by Wittig reaction of suitably protected glucosamine derivs. These lipid X analogs were recognized as substrates by the enzyme lipid A synthase and were coupled with UDP-lipid X to afford the corresponding disaccharide analog of the lipid A precursor on preparative scale. All compds. were characterized by NMR, MS, and elemental anal., and were tested for their ability to enhance nonspecific resistance to infection in mice and also for endotoxicity. The results clearly show that the new compds. express biol. activities similar to those of their O-phosphorylated natural counterparts. Furthermore, these compds. exhibit a better therapeutic index in mouse models than the std. LPS obtained from Salmonella aboptus equi.

L50 ANSWER 12 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:545024 HCAPLUS
DOCUMENT NUMBER: 113:145024
TITLE: Highly purified lipid X is devoid of immunostimulatory activity. Isolation and characterization of immunostimulating contaminants in a batch of synthetic lipid X
AUTHOR(S): Aschauer, Heinrich; Grob, Alfred; Hildebrandt, Johannes; Schuetze, Eberhard; Stuetz, Peter
CORPORATE SOURCE: Sandoz Forschungsinst., Vienna, A-1230, Austria
SOURCE: J. Biol. Chem. (1990), 265(16), 9159-64
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Lipid X, an early precursor in the biosynthesis of lipid A has been reported to directly induce cytokine release in macrophages but also to inhibit endotoxin-induced tumor necrosis factor (TNF) induction. Here, evidence is provided that these conflicting results could be due to contaminants present in different batches of lipid X used. Thus, in an apparently pure batch of cryst. lipid X as obtained by a published procedure (Macher, I., 1987) small amts. of N,O-acylated disaccharide-1-phosphates were identified. Their isolation was achieved by gel filtration on Sephadex LH-20. Further anal. of fractions showing

elevated limulus amebocyte lysate values was achieved by TLC and reverse-phase HPLC in combination with bioassays. Identification of immunostimulatory byproducts was possible by testing HPLC-fractions for TNF-induction in bone marrow-derived mouse macrophages. A disaccharide-1-phosphate contg. 4 3(R)-hydroxymyristic acids at positions, 2, 3, 2', 3', was identified as the main immunostimulatory side product. Two isomeric hydrolysis products of this compd. with only 3(R)-hydroxymyristic acid moieties attached to the disaccharide-1-phosphate were also identified. These compds. behave quite differently in the TNF induction test. The disaccharide-1-phosphate, acylated at positions 2, 2', 3', is a very potent inducer of TNF-release whereas the corresponding isomer contg. the 3(R)-hydroxymyristic acids in positions 2, 3, 2', does not induce TNF release, but strongly inhibits TNF release as induced by the former compd. Thus, contamination of pure lipid X with immunostimulatory or immunoinhibitory impurities may explain the divergent pharmacol. profiles which were attributed to synthetic lipid X.

L50 ANSWER 13 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:508906 HCAPLUS
DOCUMENT NUMBER: 113:108906
TITLE: Lipid A, the immunostimulatory principle of lipopolysaccharides?
AUTHOR(S): Loppnow, H.; Duerrbaum, I.; Brade, H.; Dinarello, C. A.; Kusumoto, S.; Rietschel, E. T.; Flad, H. D.
CORPORATE SOURCE: Forschungsinst. Borstel, Borstel, D-2061, Fed. Rep. Ger.
SOURCE: Adv. Exp. Med. Biol. (1990), 256(Endotoxin), 561-6
CODEN: AEMBAP; ISSN: 0065-2598
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lipid A has been found to be an inducer of interleukin 1 with similar potency as lipopolysaccharide (LPS). Partial structures of lipid A or core oligosaccharides are less active or not active at all. It is proposed that lipid A is the structure responsible for induction of immunostimulatory or immunoregulatory properties of LPS.

L50 ANSWER 14 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:196168 HCAPLUS
DOCUMENT NUMBER: 112:196168
TITLE: Endotoxic properties of chemically synthesized lipid A analogs. Studies on six inflammatory reactions in vivo, and one reaction in vitro
AUTHOR(S): Yoshida, Masao; Hirata, Michimasa; Inada, Katsuya; Tsunoda, Nobuko; Kirikae, Teruo; Onodera, Tsuyoshi; Ishikawa, Yoshihito; Sasaki, Osamu; Shiba, Tetsuo; et al.
CORPORATE SOURCE: Sch. Med., Iwate Med. Univ., Morioka, 020, Japan
SOURCE: Microbiol. Immunol. (1989), 33(10), 797-810
CODEN: MIIMDV; ISSN: 0385-5600
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Biol. activities of two groups of synthesized lipid A analogs, the counterpart of biosynthetic precursor, Lehmann's Ia type, 406, and E. coli lipid A type, 506, as well as their non-phosphorylated, and mono-phosphorylated analogs were investigated. The activities employed included four bone marrow cell reactions in mice, mice skin reaction, leukocytes migration in rabbits' cornea, and hemagglutination. Compd. 406 and 506 elicited bone marrow reactions in mice and hemagglutination of mouse RBC, although 406 failed to elicit hemorrhage and necrosis also in mice skin. Compd. 406 did not elicit corneal reaction in rabbits. The

results suggest that for elicitation of this reaction and mice skin reaction, acyloxyacyl structure is required. Cytotoxicity and thromboplastin prodn. of four bone marrow reactions had been previously reported to be endotoxic reactions, since these had not been elicited by peptidoglycan of Lactobacillus and Staphylococcus and 300 series synthesized analogs which did not have endotoxic structures. From these results, it seems that these two marrow reactions and hemagglutination require, as does the limulus test, the lipid A part structure as is present in 406.

L50 ANSWER 15 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:176617 HCAPLUS
DOCUMENT NUMBER: 112:176617
TITLE: Binding characteristics and cross-reactivity of three different antilipid A monoclonal antibodies
AUTHOR(S): Erich, Tineke; Schellekens, Joop; Bouter, Ally; Van Kranen, Joost; Brouwer, Ellen; Verhoef, Jan
CORPORATE SOURCE: Eijkman-Winkler Lab. Microbiol., Univ. Utrecht, Utrecht, 3584 CX, Neth.
SOURCE: J. Immunol. (1989), 143(12), 4053-60
CODEN: JOIMA3; ISSN: 0022-1767
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A detailed characterization of binding specificity and cross-reactivity of 3 antilipid A murine mAb was performed. Binding characteristics of these 3 mAb were investigated against antigen (Ag) (ReLPS, lipid A, derivs. of lipid A) in solid phase (ELISA) and in fluid phase (C consumption, inhibition studies), and upon incorporation in membranes (E: passive hemolysis assay, and liposomes: inhibition studies). Cross-reactivity with heterologous Ag was investigated in ELISA (LPS, Gram-neg. bacteria) and immunoblot expts. (LPS). The binding specificity of mAb 26-5 (IgG2b), raised against synthetic lipid A, was located in the hydrophilic region of biphospholipid A and was also exposed after membrane incorporation of lipid A or after preincubation of lipid A with polymyxin B (PMX). The mAb 26-20 (IgM), also raised against synthetic lipid A, showed binding specificity for the hydrophobic region of lipid A: no binding to membrane-assocd. lipid A could be demonstrated, and binding in ELISA could be blocked very efficiently by PMX. The reaction pattern of mAb 8-2 (IgM), raised against the heat-killed Re mutant of Salmonella typhimurium, was in part similar to that of mAb 26-20. However, inhibition of binding with PMX was less efficient and a high specificity for ReLPS, also after membrane incorporation of this Ag, was demonstrated. In contrast to mAb 26-5 and 26-20, mAb 8-2 showed extensive cross-reactivity with heterologous LPS prepns. and heat-killed as well as live Gram-neg. bacteria. Thus, each of the 3 mAb binds to a different antigenic epitope in lipid A and exposure of those epitopes for antibody binding is restricted in a differential manner, depending on mode of Ag presentation. These defined reaction patterns provide a basis for the interpretation of potential inhibitory effects on in vitro and in vivo biol. (and toxic) activities of endotoxins and Gram-neg. bacteria.

L50 ANSWER 16 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:209090 HCAPLUS
DOCUMENT NUMBER: 110:209090
TITLE: Biosynthesis of lipid A in Escherichia coli: identification of UDP-3-O-[(R)-3-hydroxymyristoyl]-.alpha.-D-glucosamine as a precursor of UDP-N2,O3-bis[(R)-3-hydroxymyristoyl]-.alpha.-D-glucosamine
AUTHOR(S): Anderson, Matt S.; Robertson, Andrew D.; Macher,

Ingolf; Raetz, Christian R. H.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: Biochemistry (1988), 27(6), 1908-17
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The lipid A disaccharide of the E. coli envelope is synthesized from 2 fatty acylated glucosamine derivs.: UDP-N2,O3-bis[(R)-3-hydroxytetradecanoyl]-.alpha.-D-glucosamine (I) and N2,O3-bis[(R)-3-hydroxytetradecanoyl]-.alpha.-D-glucosamine 1-phosphate (II). It is known that I is generated in exts. of E. coli by fatty acylation of UDP-GlcNAc, giving UDP-3-O-[(R)-3-hydroxymyristoyl]-GlcNAc (III) as the 1st intermediate, which is rapidly converted to I. A novel enzyme is now demonstrated in the cytoplasmic fraction of E. coli, capable of deacylating III to form UDP-3-O-[(R)-3-hydroxymyristoyl]glucosamine (IV). The covalent structure of the previously underscribed IV intermediate was established by 1H NMR spectroscopy and fst atom bombardment mass spectrometry. This material can be made to accumulate in E. coli exts. upon incubation of III in the absence of the fatty acyl donor [(R)-3-hydroxymyristoyl]-acyl carrier protein. However, addn. of the isolated deacetylation product IV back to membrane-free exts. of E. coli in the presence of [(R)-3-hydroxymyristoyl]-acyl carrier protein results in rapid conversion of this compd. into the more hydrophobic products I, II, and O-[2-amino-2-deoxy-N2,O3-bis[(R)-3-hydroxytetradecanoyl]-.beta.-D-glucopyranosyl]-(1.fwdarw.6)-2-amino-2-deoxy-N2,O3-bis[(R)-3-hydroxytetradecanoyl]-.alpha.-D-glucopyranose 1-phosphate (tetraacyldisaccharide-1-P), demonstrating its competency as a precursor. In vitro incubations using [3H]acetyl-III confirmed release of the acetyl moiety in this system as acetate, not as some other acetyl deriv. The deacetylation reaction was inhibited by 1 mM N-ethylmaleimide, while the subsequent N-acylation reaction was not. These observations provide strong evidence that IV is a true intermediate in the biosynthesis of I and lipid A.

L50 ANSWER 17 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:628195 HCAPLUS
DOCUMENT NUMBER: 109:228195
TITLE: Structural requirements of lipid A species in activation of clotting enzymes from the horseshoe crab, and the human complement cascade
AUTHOR(S): Takada, Haruhiko; Kotani, Shozo; Tanaka, Shigenori; Ogawa, Tomohiko; Takahashi, Ichiro; Tsujimoto, Masachika; Komuro, Tetsuo; Shiba, Tetsuo; Kusumoto, Shoichi; et al.
CORPORATE SOURCE: Dent. Sch., Osaka Univ., Osaka, 565, Japan
SOURCE: Eur. J. Biochem. (1988), 175(3), 573-80
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The structure/activity relationship of lipid A, a bioactive center of endotoxic lipopolysaccharides, in the activation of the clotting enzyme cascade of a horseshoe crab amebocyte lysate (Limulus activity) and the complement system in human serum, was examd. using synthetic lipids A and related compds. Regarding Limulus activity, a newly developed colorimetric method, which utilizes a mixt. of recombined clotting factors and a chromogenic substance, was much more sensitive for detecting changes in the chem. structure of test compds. than the conventional gelation method using the amebocyte whole lysate. (.beta.1-6)-D-Glucosamine phosphates, which in structure correspond or are analogous to the

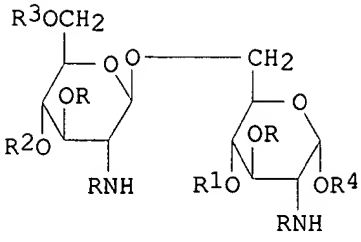
non-reducing or reducing moieties of lipids A and biosynthetic disaccharide lipid A precursors, showed only negligible activity in the colorimetric tests, but they exhibited a distinct though much weaker gelation activity than the parent disaccharide mols. The assay results obtained by the colorimetric Limulus test correlate better with the pyrogenicity of the test synthetic compds. than those given by the gelation method, although the dependence of pyrogenicity on chem. structure is greater. The presence of 3-hydroxyacyl groups on the bisphosphorylated (.beta.1-6)-D-glucosamine disaccharide backbone is the prerequisite for effective activation of the clotting enzyme cascade of horseshoe crab amebocyte lysate, while the presence of an adequate no. (one or two) of 3-acyloxyacyl groups on the disaccharide bisphosphate backbone is needed for full pyrogenicity. Complement activation, on the other hand, showed structural requirements quite different from those for the colorimetric Limulus activity and the pyrogenicity. The disaccharide compds. that had only non-hydroxylated acyl groups, acylated glucosamine phosphates that had the structure of the non-reducing portion of lipids A and biosynthetic disaccharide precursors, which were scarcely active in the colorimetric Limulus test, caused complement activation comparable to or sometimes stronger than that of the parent disaccharide mols. Acylglucosamine phosphates, corresponding in structure to the reducing moiety of disaccharide compds., however, showed little activity.

L50 ANSWER 18 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:625538 HCAPLUS
DOCUMENT NUMBER: 109:225538
TITLE: Intracellular serine-protease zymogen, factor C, from horseshoe crab hemocytes. Its activation by synthetic lipid A analogs and acidic phospholipids
AUTHOR(S): Nakamura, Takanori; Tokunaga, Fuminori; Morita, Takashi; Iwanaga, Sadaaki; Kusumoto, Shoichi; Shiba, Tetsuo; Kobayashi, Tetsuyuki; Inoue, Keizo
CORPORATE SOURCE: Fac. Sci., Kyushu Univ., Fukuoka, 812, Japan
SOURCE: Eur. J. Biochem. (1988), 176(1), 89-94
CODEN: EJBCAI; ISSN: 0014-2956
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An intracellular clotting factor, factor C, found in horseshoe crab (Tachypleus tridentatus) hemocytes is a lipopolysaccharide-sensitive serine protease zymogen, which participates in the initiation of the hemolymph clotting system. The subsequent study of this zymogen, using various synthetic lipid A analogs, revealed that the zymogen factor C is rapidly activated by acylated (.beta.1-6)-D-glucosamine disaccharide bisphosphate (synthetic Escherichia cell-type lipid A, and the corresponding 4'-monophosphate analogs. However, the corresponding nonphosphorylated lipid A did not activate factor C, indicating that a phosphate ester group linked with the (.beta.1-6)-D-glucosamine disaccharide backbone is required for the zymogen activation. During these studies it was also found that the zymogen factor C is significantly activated by acidic phospholipids, such as phosphatidylinositol, phosphatidylglycerol, and cardiolipin, but not at all by neutral phospholipids. The rate of this activation, however, was affected markedly by ionic strength in the reaction mixt., although such an effect was not obsd. in the lipid A-mediated activation of factor C. A variety of neg. charged surfaces, such as sulfatide, dextran sulfate and ellagic acid, which are known as typical initiators for activation of the mammalian intrinsic clotting system, did not show any effect on the zymogen factor C activation. These results suggest that lipid A is the most effective trigger to initiate the activation of the horseshoe crab hemolymph clotting system.

L50 ANSWER 19 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:204944 HCAPLUS
DOCUMENT NUMBER: 108:204944
TITLE: Chemical synthesis of a biosynthetic precursor of lipid A with a phosphorylated tetraacyl disaccharide structure
AUTHOR(S): Imoto, Masahiro; Yoshimura, Hiroyuki; Yamamoto, Michiharu; Shimamoto, Tetsuo; Kusumoto, Shoichi; Shiba, Tetsuo
CORPORATE SOURCE: Fac. Sci., Osaka Univ., Toyonaka, 560, Japan
SOURCE: Bull. Chem. Soc. Jpn. (1987), 60(6), 2197-204
CODEN: BCSJA8; ISSN: 0009-2673
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 108:204944
GI For diagram(s), see printed CA Issue.
AB Glucosamine disaccharide diphosphate [I; R = (R)-3-hydroxytetradecanoyl throughout; R1 = R2 = P(O)(OH)2] was prepd. via prepn. of the .beta.-(1.fwdarw.6)-disaccharide without long chain acyl groups, introduction of 3-(benzyloxy)tetradecanoyl groups onto the 2 amino and 2 OH groups, phosphorylation of the 4'-position, phosphorylation of the glycosidic position, and hydrogenolytic deprotection. The monophosphates I [R1 = P(O)(OH)2, R2 = H; R1 = H, R2 = P(O)(OH)2] and the dephospho deriv. I (R1 = R2 = H) were prepd. by slight modification of the above synthetic route. The diphosphate prepd. was identical with a natural biosynthetic precursor of lipid A which corresponds to the lipophilic part of the lipopolysaccharide (LPS) in the bacterial cell wall. The synthetic di- and monophosphates exhibited many of the typical endotoxic activities of LPS. This established the chem. structure of the biosynthetic precursor of lipid A and elucidated the fundamental structure required for the expression of these activities.

L50 ANSWER 20 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1985:214793 HCAPLUS
DOCUMENT NUMBER: 102:214793
TITLE: Immunopharmacological activities of a synthetic counterpart of a biosynthetic lipid A precursor molecule and of its analogs
AUTHOR(S): Takada, Haruhiko; Kotani, Shozo; Tsujimoto, Masachika; Ogawa, Tomohiko; Takahashi, Ichiro; Harada, Kazuhiro; Katsukawa, Chihiro; Tanaka, Shigenori; Shiba, Tetsuo; et al.
CORPORATE SOURCE: Dent. Sch., Osaka Univ., Suita, 565, Japan
SOURCE: Infect. Immun. (1985), 48(1), 219-27
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
GI

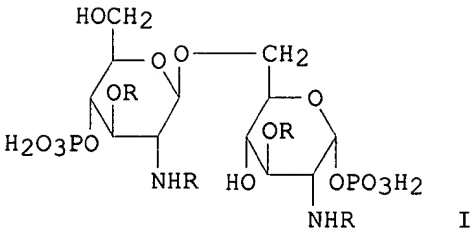


I, R=COCH2CH(OH)(CH2)10Me

AB Of the 6 synthetic lipid A analogs tested 3 (I; R1, R2, R3, R4 are H or P) showed immunopharmacol. activity in most of the in vitro test used. All of the synthetic analogs, however, were far less active than natural Escherichia coli lipid A.

L50 ANSWER 21 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1984:611613 HCAPLUS
DOCUMENT NUMBER: 101:211613
TITLE: Chemical synthesis of phosphorylated tetraacyl disaccharide corresponding to a biosynthetic precursor of lipid A
AUTHOR(S): Imoto, M.; Yoshimura, H.; Yamamoto, M.; Shimamoto, T.; Kusumoto, S.; Shiba, T.
CORPORATE SOURCE: Fac. Sci., Osaka Univ., Toyonaka, 560, Japan
SOURCE: Tetrahedron Lett. (1984), 25(25), 2667-70
CODEN: TELEAY; ISSN: 0040-4039
DOCUMENT TYPE: Journal
LANGUAGE: English
GI For diagram(s), see printed CA Issue.
AB A total synthesis of glucosamine disaccharide 1,4'-diphosphate I [RCO = Me(CH2)10CH(OH)CH2CO] is described. This if the 1st confirmation of the fundamental structure of lipid A since the synthetic compd. exhibited most of the characteristic biol. activities of natural endotoxin.

L50 ANSWER 22 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1984:528223 HCAPLUS
DOCUMENT NUMBER: 101:128223
TITLE: Immunobiologically active lipid A analogs synthesized according to a revised structural model of natural lipid A
AUTHOR(S): Kotani, Shozo; Takada, Haruhiko; Tsujimoto, Masachika; Ogawa, Tomohiko; Harada, Kazuhiro; Mori, Yoshihide; Kawasaki, Akinori; Tanaka, Atsushi; Nagao, Shigeki; et al.
CORPORATE SOURCE: Dent. Sch., Osaka Univ., Suita, 565, Japan
SOURCE: Infect. Immun. (1984), 45(1), 293-6
CODEN: INFIBR; ISSN: 0019-9567
DOCUMENT TYPE: Journal
LANGUAGE: English
GI



I

AB Synthetic lipid A analogs which have 2 amide-bound and 2 ester-bound (R)-3-hydroxytetradecanoyl groups at the C-2 and -2' and C-3 and -3' positions of .beta.(1-6)glucosamine disaccharide mono- or diphosphates showed high activities in most in vitro assays, and the lethality of a diphosphate deriv. (I; R = COCH2CH(OH)(CH2)10Me) to galactosamine-treated mice was almost comparable to that of natural lipid A. The pyrogenicity and Shwartzman induction activity of the synthetic analogs, however, were much less than those of natural lipid A.

L50 ANSWER 23 OF 23 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1984:420336 HCAPLUS
DOCUMENT NUMBER: 101:20336
TITLE: The biosynthesis of Gram-negative endotoxin. Formation of lipid A disaccharides from monosaccharide precursors in extracts of Escherichia coli
AUTHOR(S): Ray, Bryan L.; Painter, George; Raetz, Christian R. H.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Biol. Chem. (1984), 259(8), 4852-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An enzyme in the cytosol of E. coli was discovered that generates lipid A disaccharides from monosaccharide precursors by the following route: 2,3-diacyl-GlcN-1-P + UDP-2,3-diacyl-GlcN .fwdarw. 2,3-diacyl-GlcN (.beta.,1.fwdarw.6) 2,3-diacyl-GlcN-1-P + UDP. The presence, in vivo, of the precursors 2,3-diacylglucosamine 1-phosphate (2,3-diacyl-GlcN-1-P) (lipid X of E. coli) and UDP-2,3-diacylglucosamine (UDP-2,3-diacyl-GlcN) was previously shown. Both substrates are novel glucosamine-derived phospholipids, acylated with .beta.-hydroxymyristoyl moieties, and accumulate in E. coli mutants defective in the pgsB gene. Synthetic ADP-, GDP-, and CDP-2,3-diacylglucosamines are inefficient substrates as compared to the naturally occurring UDP deriv. The free-acid form of the tetraacyldisaccharide 1-phosphate product (C68H129N2O20P) that is generated in vitro has a mol. wt. of 1325.74 as judged by fast atom bombardment mass spectrometry. Mild acid hydrolysis (0.1M HCl for 30 min at 100.degree.) liberates >95% of the phosphate moiety as inorg. phosphate. Detailed anal. by 1H and 13C NMR spectroscopy confirms the presence of a phosphate residue at position 1 of the disaccharide, an .beta.-anomeric configuration at the reducing end, and a .beta.,1.fwdarw.6 linkage between the 2 glucosamines. Importantly the disaccharide 1-phosphate synthase is missing in exts. of E. coli strains harboring the pgsB1 mutation, which is consistent with the massive accumulation of 2,3-diacyl-GlcN-1-P and UDP-2,3-diacyl-GlcN in vivo. The enzymic reaction reported represents a major biosynthetic route for the formation of lipid A disaccharides in E. coli and other gram-neg. bacteria. An in vitro system for the biosynthesis of lipid A disaccharides has not been described previously.

=> d ibib abs

L15 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:360183 HCAPLUS
DOCUMENT NUMBER: 134:362273
TITLE: Genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy
INVENTOR(S): Forsyth, R. Allyn; Ohlsen, Kari; Zyskind, Judith
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 522 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034810	A2	20010517	WO 2000-US30950	20001109

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-164415 P 19991109

AB The sequences of nucleic acids encoding proteins required for E. coli proliferation are disclosed. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate mols. for rational drug discovery programs. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than E. coli. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms as well as to screen for antimicrobial agents.

=> d ind

L15 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
IC ICM C12N015-31
ICS C12N015-11; C12N015-10; C07K014-245
CC 3-3 (Biochemical Genetics)
Section cross-reference(s): 6, 10
ST sequence Escherichia proliferation assocd gene protein; antimicrobial screening Escherichia proliferation assocd gene protein
IT Genetic vectors
(E. coli proliferation-assocd. gene on; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)
IT Ribozymes
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(anti-proliferation-assocd. genes; genes essential for microbial

proliferation and their use for antimicrobial screening or in antisense therapy)

IT Antibodies
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(anti-proliferation-assocd. protein; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT Nucleic acids
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(antisense, to proliferation-assocd. genes; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT Antibiotics
Antimicrobial agents
Growth, microbial
(genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT Escherichia coli
(genes involved in proliferation of; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT DNA sequences
(of proliferation-assocd. genes of Escherichia coli)

IT Molecular cloning
(of proliferation-assocd. genes; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT Protein sequences
(of protein products of proliferation-assocd. genes of Escherichia coli)

IT Animal cell
Aspergillus
Aspergillus fumigatus
Bacillus (bacterium genus)
Bacillus anthracis
Bacteria (Eubacteria)
Campylobacter
Campylobacter jejuni
Candida
Candida albicans
Chlamydia
Chlamydia pneumoniae
Chlamydia trachomatis
Clostridium
Clostridium botulinum
Cryptococcus (fungus)
Cryptococcus neoformans
Enterobacter
Enterobacter cloacae
Enterococcus
Enterococcus faecalis
Escherichia
Fungi
Gram-negative bacteria
Haemophilus
Haemophilus influenzae
Helicobacter
Helicobacter pylori
Klebsiella

Klebsiella pneumoniae
Mycobacterium
Mycobacterium leprae
Mycobacterium tuberculosis
Neisseria
Neisseria gonorrhoeae
Plant cell
Pseudomonas
Pseudomonas aeruginosa
Saccharomyces
Saccharomyces cerevisiae
Salmonella
Salmonella choleraesuis
Salmonella paratyphi
Salmonella typhi
Salmonella typhimurium
Staphylococcus
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus
Streptococcus pneumoniae
Treponema
Treponema pallidum
Yersinia
Yersinia pestis
(proliferation-assocd. gene expression in; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

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RL: ARG (Analytical reagent use); BPN (Biosynthetic preparation); PRP
(Properties); ANST (Analytical study); BIOL (Biological study); PREP
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(amino acid sequence; genes essential for microbial proliferation and
their use for antimicrobial screening or in antisense therapy)

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(Escherichia coli gene **lpxB**) 113535-81-2, DNA (Escherichia
coli gene dnaE) 113670-29-4, DNA (Escherichia coli gene hsdM)
118901-13-6, DNA (Escherichia coli gene **lpxA**) 127884-37-1, DNA
(Escherichia coli strain K-12 280-amino acid protein gene) 129652-91-1,
DNA (Escherichia coli gene cyoA) 129652-92-2, DNA (Escherichia coli gene
cyoB) 129652-93-3, DNA (Escherichia coli gene cyoC) 129652-94-4, DNA
(Escherichia coli gene cyoD) 129652-95-5, DNA (Escherichia coli gene
cyoE) 134711-83-4, DNA (Escherichia coli clone pJC765 gene xprB)
136395-69-2, DNA (insertion sequence IS1B clone 5A5 gene insA)

205901-49-1, DNA (Escherichia coli gene pfs) 286446-06-8 286446-07-9, DNA (Escherichia coli gene yfdH) 286446-08-0, DNA (Escherichia coli gene yfdI) 286446-14-8, DNA (Escherichia coli gene yegN) 286446-15-9, DNA (Escherichia coli gene yegO) 286446-16-0, DNA (Escherichia coli gene yegB) 286446-43-3 286446-44-4, DNA (Escherichia coli gene rplO) 286446-45-5, DNA (Escherichia coli gene rpsE) 286446-46-6, DNA (Escherichia coli gene rplR) 286446-47-7, DNA (Escherichia coli gene rplF) 286446-48-8, DNA (Escherichia coli gene rpsH) 286446-49-9, DNA (Escherichia coli gene rpsN) 286446-50-2, DNA (Escherichia coli gene rplE) 286446-51-3, DNA (Escherichia coli gene rplX) 286446-52-4, DNA (Escherichia coli gene rplN) 286447-10-7, DNA (Escherichia coli gene rplB) 286447-14-1, DNA (Escherichia coli gene rpmI) 286447-15-2, DNA (Escherichia coli gene infC) 286447-16-3, DNA (Escherichia coli gene thrS) 300756-87-0, DNA (Escherichia coli gene yrfI) 339372-90-6, DNA (Escherichia coli gene ycfS) 339372-91-7, DNA (Escherichia coli gene arp) 339372-92-8, DNA (Escherichia coli gene ypjA) 340046-01-7, DNA (Escherichia coli gene b2269) 340046-02-8, DNA (Escherichia coli gene dgoA) 340046-03-9, DNA (Escherichia coli gene dgoK) 340046-04-0, DNA (Escherichia coli gene yidW) 340046-05-1, DNA (Escherichia coli gene b3694) 340046-06-2, DNA (Escherichia coli gene xylF) 340046-07-3, DNA (Escherichia coli gene yhfL) 340046-08-4, DNA (Escherichia coli gene yhfM) 340046-09-5, DNA (Escherichia coli gene yhfN) 340046-10-8, DNA (Escherichia coli gene yhfO) 340046-11-9, DNA (Escherichia coli gene ybcQ) 340046-12-0, DNA (Escherichia coli gene ffh) 340046-13-1, DNA (Escherichia coli gene recJ) 340046-14-2, DNA (Escherichia coli gene dsbC) 340046-15-3, DNA (Escherichia coli gene ecpD) 340046-16-4, DNA (Escherichia coli gene htrE) 340046-17-5, DNA (Escherichia coli gene yciR) 340046-18-6, DNA (Escherichia coli gene sfmC) 340046-19-7, DNA (Escherichia coli gene sfmD) 340046-20-0, DNA (Escherichia coli gene sfmH) 340046-21-1, DNA (Escherichia coli gene sfmF) 340046-22-2, DNA (Escherichia coli gene yceH) 340046-23-3, DNA (Escherichia coli gene mviM) 340046-24-4, DNA (Escherichia coli gene sanA) 340046-25-5, DNA (Escherichia coli gene b2145) 340046-26-6, DNA (Escherichia coli gene ycgB) 340046-27-7, DNA (Escherichia coli gene yedV) 340046-28-8, DNA (Escherichia coli gene yedW) 340046-29-9, DNA (Escherichia coli gene b2107) 340046-30-2, DNA (Escherichia coli gene b2106) 340046-31-3, DNA (Escherichia coli gene hybG) 340046-32-4, DNA (Escherichia coli gene hybF) 340046-33-5, DNA (Escherichia coli gene hybE) 340046-34-6, DNA (Escherichia coli gene hybD) 340046-35-7, DNA (Escherichia coli gene hybC) 340046-36-8, DNA (Escherichia coli gene hybB) 340046-37-9, DNA (Escherichia coli gene hybA) 340046-38-0, DNA (Escherichia coli gene hrpB) 340046-39-1, DNA (Escherichia coli gene b1399) 340046-40-4, DNA (Escherichia coli gene b1400) 340046-41-5, DNA (Escherichia coli gene agaZ) 340046-42-6, DNA (Escherichia coli gene agaV) 340046-43-7, DNA (Escherichia coli gene agaW) 340046-44-8, DNA (Escherichia coli gene agaA) 340046-45-9, DNA (Escherichia coli gene agaS) 340046-46-0, DNA (Escherichia coli gene agaY) 340046-47-1, DNA (Escherichia coli gene rpsK) 340046-48-2, DNA (Escherichia coli gene yehW) 340046-49-3, DNA (Escherichia coli gene yehX) 340046-50-6, DNA (Escherichia coli gene yehY) 340046-51-7, DNA (Escherichia coli gene yehZ) 340046-52-8, DNA (Escherichia coli gene yadS) 340046-53-9, DNA (Escherichia coli gene yadT) 340046-54-0, DNA (Escherichia coli gene rnhB) 340046-55-1, DNA (Escherichia coli gene ykgE) 340046-56-2, DNA (Escherichia coli gene ykgF) 340046-57-3, DNA (Escherichia coli gene ykgG) 340046-58-4, DNA (Escherichia coli gene b1497) 340046-59-5, DNA (Escherichia coli gene b1498) 340046-60-8, DNA (Escherichia coli gene yfjW) 340046-61-9, DNA (Escherichia coli gene b2758) 340046-62-0, DNA (Escherichia coli gene ygcM) 340046-63-1, DNA (Escherichia coli gene ygcN) 340046-64-2, DNA (Escherichia coli gene b2767) 340046-65-3, DNA (Escherichia coli gene b2768) 340046-66-4, DNA (Escherichia coli gene yhcB) 340046-67-5, DNA

(Escherichia coli gene hhoA) 340046-68-6, DNA (Escherichia coli gene hhoB) 340046-69-7, DNA (Escherichia coli gene yihK) 340046-70-0, DNA (Escherichia coli gene adi) 340046-71-1, DNA (Escherichia coli gene adiY) 340046-72-2, DNA (Escherichia coli gene yjhB) 340046-73-3, DNA (Escherichia coli gene yjhC) 340046-74-4, DNA (Escherichia coli gene hsdS) 340046-75-5, DNA (Escherichia coli gene b1357) 340046-76-6, DNA (Escherichia coli gene b1358) 340046-77-7, DNA (Escherichia coli gene ydaU) 340046-78-8, DNA (Escherichia coli gene b1360) 340046-79-9, DNA (Escherichia coli gene b1361) 340046-80-2, DNA (Escherichia coli gene b1362) 340046-81-3, DNA (Escherichia coli gene ybbQ) 340046-82-4, DNA (Escherichia coli gene ybbV) 340046-83-5, DNA (Escherichia coli gene b0511) 340046-84-6, DNA (Escherichia coli gene yegM) 340046-85-7, DNA (Escherichia coli gene yigK) 340046-86-8, DNA (Escherichia coli gene modA) 340046-87-9, DNA (Escherichia coli gene modB) 340046-88-0, DNA (Escherichia coli gene modC) 340046-89-1, DNA (Escherichia coli gene ynaF) 340046-90-4, DNA (Escherichia coli gene b1377) 340046-91-5, DNA (Escherichia coli gene ppdB) 340046-92-6, DNA (Escherichia coli gene ppdA) 340046-93-7, DNA (Escherichia coli gene yrfF) 340046-94-8, DNA (Escherichia coli gene yrfG) 340046-95-9, DNA (Escherichia coli gene yrfH) 340046-96-0, DNA (Escherichia coli gene b2353) 340046-97-1, DNA (Escherichia coli gene ygeF) 340046-98-2, DNA (Escherichia coli gene insB3) 340046-99-3, DNA (Escherichia coli gene rhsA) 340047-00-9, DNA (Escherichia coli gene yibJ)
RL: ARG (Analytical reagent use); BPR (Biological process); PRP (Properties); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT 339371-64-1 339371-65-2 339371-66-3 339371-67-4, DNA (Escherichia coli gene arp fragment) 339371-68-5, DNA (Escherichia coli gene arp fragment) 339371-69-6 339371-70-9 339371-71-0 339371-72-1
339371-73-2 339371-74-3, DNA (Escherichia coli gene rep fragment)
339371-75-4 339371-76-5 339371-77-6 339371-78-7 339371-79-8
339371-80-1 339371-81-2 339371-82-3 339371-83-4 339371-84-5
339371-85-6 339371-86-7 339371-87-8 339371-88-9 339371-89-0
339371-90-3 339371-91-4 339371-92-5 339371-93-6 339371-94-7
339371-95-8 339371-96-9 339371-97-0 339371-98-1 339371-99-2, DNA (Escherichia coli gene ffh fragment) 339372-00-8 339372-01-9
339372-02-0 339372-03-1 339372-04-2 339372-05-3 339372-06-4
339372-07-5 339372-08-6 339372-09-7 339372-10-0 339372-11-1
339372-12-2 339372-13-3 339372-14-4 339372-15-5 339372-16-6
339372-17-7 339372-18-8 339372-19-9 339372-20-2 339372-21-3
339372-22-4 339372-23-5 339372-24-6 339372-25-7 339372-26-8
339372-27-9 339372-28-0 339372-29-1 339372-30-4 339372-31-5
339372-32-6 339372-33-7 339372-34-8 339372-35-9 339372-36-0
339372-37-1 339372-38-2 339372-39-3 339372-40-6 339372-41-7
339372-42-8 339372-43-9 339372-44-0 339372-45-1 339372-46-2
339372-47-3 339372-48-4 339372-49-5 339372-50-8 339372-51-9
339372-52-0 339372-53-1 339372-54-2 339372-55-3 339372-56-4
339372-57-5 339372-58-6 339372-59-7 339372-60-0 339372-61-1
339372-62-2 339372-63-3 339372-64-4 339372-65-5 339372-66-6
339372-67-7 339372-68-8 339372-69-9 339372-70-2 339372-71-3
339372-72-4 339372-73-5 339372-74-6 339372-75-7 339372-76-8
339372-77-9 339372-78-0 339372-79-1 339372-80-4 339372-81-5
339372-82-6 339372-83-7 339372-84-8 339372-85-9 339372-86-0
339372-87-1 339372-88-2 339372-89-3
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(nucleotide sequence; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

IT 286447-26-5 286450-22-4
RL: PRP (Properties)
(unclaimed sequence; genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy)

4,895,660
5,561,064

=> d ibib abs

L14 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:429591 HCAPLUS
DOCUMENT NUMBER: 127:49213
TITLE: Novel non-pyrogenic bacterial strains and use of the same
INVENTOR(S): Hone, David M.; Powell, Robert J.
PATENT ASSIGNEE(S): University of Maryland At Baltimore, USA; Hone, David M.; Powell, Robert J.
SOURCE: PCT Int. Appl., 124 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9718837	A1	19970529	WO 1996-US19875	19961122
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9722784	A1	19970611	AU 1997-22784	19961122
EP 841941	A1	19980520	EP 1996-945937	19961122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 5997881	A	19991207	US 1997-802371	19970219
PRIORITY APPLN. INFO.:			US 1995-7478	19951122
			WO 1996-US19875	19961122

AB The present invention provides **gram-neg.** bacterial strains that produce substantially pure non-pyrogenic lipopolysaccharide or lipid A. The present invention also relates to a use of said strains for the prepn. of non-pyrogenic DNA and use of the same for introducing endogenous or foreign genes into animal cells or animal tissue. Further, the present invention relates to a use of said strains for the prepn. of non-pyrogenic bacterial proteins and polysaccharides antigens for use as **vaccines**.

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L14 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2002 ACS
IC ICM A61K039-02
ICS A61K048-00; C12P021-06; C12P019-00; C12P019-34; C12P001-04; C12N015-00; C12N001-12; C12N001-20; C07H021-02
CC 15-2 (Immunochemistry)
Section cross-reference(s): 3, 10
ST nonpyrogenic bacteria lipopolysaccharide antigen **vaccine** DNA
IT Pilins
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(CFA (colonization factor antigen), enterotoxigenic Escherichia coli; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT **Vaccines**
(antigen; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Mutation
(auxotrophic; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Peptides, biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biol. active; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Bird (Aves)
Fish
Mammal (Mammalia)
Reptile
(cell; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Proteins (general), biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cochelates; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Virulence (microbial)
(factor; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(htrB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kUsA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdsA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdsB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdtA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxA**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into

animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxB**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(lpxC; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxD**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Cell cycle
(modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(msbB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Aeromonas
Animal cells
Animal tissue
Bacteriophage
Bordetella
Brucella
Campylobacter
Chlamydia
Citrobacter
Corynebacterium
Cosmids
Escherichia
Eukaryote (Eukaryotae)
Francisella
Gram-negative bacteria
Haemophilus
Helicobacter
Immunomodulators
Klebsiella
Legionella
Liposomes
Neisseria
Pathogenic microorganism
Phagemids
Plasmids
Pseudomonas
Rhodobacter
Salmonella
Salmonella typhimurium
Shigella
Staphylococcus
Streptococcus
Therapy

Vibrio
Yersinia
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Antisense RNA
Genetic elements
Interferon .alpha.
Interferon .beta.
Interferon .gamma.
Interleukin 1
Interleukin 10
Interleukin 11
Interleukin 12
Interleukin 13
Interleukin 2
Interleukin 3
Interleukin 4
Interleukin 5
Interleukin 6
Interleukin 7
Interleukin 8
Interleukin 9
Monoclonal antibodies
O antigen
Ribozymes
Tumor necrosis factor .alpha.
gp120 (env glycoprotein)
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Calmodulins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Estrogens
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Quaternary ammonium compounds, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Lipid A
Lipopolysaccharides
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Plasmids
(pJGX15C; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Metazoan (Metazoa)
Protozoa
Virus
(pathogen; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or

tissue)

IT Stress (animal)
(response modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Phenotypes
(restriction modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ssc; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Polysaccharides, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(surface; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT DNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(topol.; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT Antigens
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**vaccine**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT 9031-11-2, .beta.-Galactosidase
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

IT 57-83-0, Progesterone, biological studies 64-20-0D, Tetramethylammonium bromide, tetraacyl derivs. 141-43-5, biological studies 1406-11-7, Polymyxin 2390-68-3 9001-29-0, Factor X 9002-72-6, Growth hormone 9004-10-8, Insulin, biological studies 9035-81-8, Antitrypsin 9061-61-4, Nerve growth factor 11096-26-7, Erythropoietin 20064-29-3D, 1,2-Diacyl deriv. 25104-18-1, Polylysine 62229-50-9, Epidermal growth factor 81627-83-0, M-CSF 83869-56-1, GM-CSF 104162-48-3 168479-03-6
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery **vaccine** genes or DNA into animal cell or tissue)

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L39 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:360183 HCAPLUS
DOCUMENT NUMBER: 134:362273
TITLE: Genes essential for microbial proliferation and their use for antimicrobial screening or in antisense therapy
INVENTOR(S): Forsyth, R. Allyn; Ohlsen, Kari; Zyskind, Judith
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 522 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001034810	A2	20010517	WO 2000-US30950	20001109

W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-164415 P 19991109
AB The sequences of nucleic acids encoding proteins required for E. coli proliferation are disclosed. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate mols. for rational drug discovery programs. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than E. coli. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms as well as to screen for antimicrobial agents.

L39 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:41310 HCAPLUS
DOCUMENT NUMBER: 135:165814
TITLE: Gram-negative bacteria induce proinflammatory cytokine production by monocytes in the absence of lipopolysaccharide (LPS)
AUTHOR(S): Uronen, H.; Williams, A. J.; Dixon, G.; Andersen, S. R.; Van Der Ley, P.; Van Deuren, M.; Callard, R. E.; Klein, N.
CORPORATE SOURCE: Immunobiology Unit, Institute of Child Health, University College London, London, WC1N 1EH, UK
SOURCE: Clin. Exp. Immunol. (2000), 122(3), 312-315
CODEN: CEXIAL; ISSN: 0009-9104
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Tumor necrosis factor-alpha (TNF-.alpha.), IL-1.alpha. and IL-6 prodn. by

human monocytes in response to a clin. strain of the Gram-neg. encapsulated bacteria Neisseria meningitidis and an isogenic **lpxA** - strain deficient in LPS was investigated. Wild-type N. meningitidis at concns. between 105 and 108 organisms/mL and purified LPS induced proinflammatory cytokine prodn. High levels of these cytokines were also produced in response to the **lpxA**- strain at 107 and 108 organisms/mL. The specific LPS antagonist bactericidal/permeability-increasing protein (rBPI21) inhibited cytokine prodn. induced by LPS and wild-type bacteria at 105 organisms/mL but not at higher concns., and not by LPS-deficient bacteria at any concn. These data show that proinflammatory cytokine prodn. by monocytes in response to N. meningitidis does not require the presence of LPS. Therapeutic strategies designed to block LPS alone may not therefore be sufficient for interrupting the inflammatory response in severe meningococcal disease.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L39 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:166730 HCAPLUS
DOCUMENT NUMBER: 130:207230
TITLE: Viable Lipid A-deficient mutants of Gram negative mucosal bacteria and their use in the development of vaccines
INVENTOR(S): Van Der Ley, Peter Andre; Steeghs, Liana Juliana Josephine Margret
PATENT ASSIGNEE(S): De Staat Der Nederlanden, Vertegenwoordigd Door De Minister Van Welzijn, Vol, Neth.
SOURCE: PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9910497	A1	19990304	WO 1997-NL474	19970821
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9739540	A1	19990316	AU 1997-39540	19970821
EP 991761	A1	20000412	EP 1997-936881	19970821
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP 2001514001	T2	20010911	JP 2000-507805	19970821
NO 2000000774	A	20000414	NO 2000-774	20000217

PRIORITY APPLN. INFO.: WO 1997-NL474 A 19970821

AB It is possible to inactivate the early stage of lipid A synthesis of mucosal gram neg. bacteria without compromising cell viability. In particular the **lpxA** mutants of Neisseria meningitidis were found to be completely lipopolysaccharide(LPS)-deficient. The major outer membrane proteins (OMPs) were detected in normal amts. The finding provides important implications for understanding of structure and biogenesis of the outer membrane. On a practical level, the availability of LPS-deficient mutants of pathogenic mucosal bacteria such as N. meningitidis opens up new avenues to vaccine development. It enables easy

isolation of endotoxin-free purified proteins, outer membranes or even whole-cell prepns. for use in immunization.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L39 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:535707 HCAPLUS
DOCUMENT NUMBER: 127:245223
TITLE: Shortened hydroxyacyl chains on lipid A of Escherichia coli cells expressing a foreign UDP-N-acetylglucosamine O-acyltransferase
AUTHOR(S): Odegaard, Timna J.; Kaltashov, Igor A.; Cotter, Robert J.; Steeghs, Liana; Van Der Ley, Peter; Khan, Shahid; Maskell, Duncan J.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1997), 272(32), 19688-19696
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The first reaction of lipid A biosynthesis in Gram neg. bacteria is catalyzed by UDP-N-acetylglucosamine (UDP-GlcNAc) O-acyltransferase, the product of the **lpxA** gene. The reaction involves the transfer of an acyl chain from hydroxyacyl-acyl carrier protein (ACP) to the glucosamine 3-OH position of UDP-GlcNAc. The lipid A isolated from Escherichia coli contains (R)-3-hydroxymyristate at the 3 and 3' positions. Accordingly, **LpxA** of E. coli is highly selective for (R)-3-hydroxymyristoyl-ACP over ACP thioesters of longer or shorter acyl chains. We now demonstrate that the **lpxA** gene from Neisseria meningitidis encodes a similar acyltransferase that selectively utilizes 3-hydroxylauroyl-ACP. Strains of E. coli harboring the temp.-sensitive lpxA2 mutation make very little lipid A and lose viability rapidly at 42.degree.C. We have created an E. coli strain in which the chromosomal lpxA2 mutation is complemented by the N. meningitidis **lpxA** gene introduced on a plasmid. This strain, RO138/pTO6, grows similarly to wild type cells at 42.degree.C and produces wild type levels of lipid A. However, the lipid A isolated from RO138/pTO6 contains mostly hydroxylaurate and hydroxydecanoate in the 3 and 3' positions. The strain RO138/pTO6 is more susceptible than wild type to certain antibiotics at 42.degree.C. This is the first report of an E. coli strain growing with shortened hydroxyacyl chains on its lipid A. The **lpxA** gene product appears to be a crit. determinant of the length of the ester-linked hydroxyacyl chains found on lipid A in living cells.

L39 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:429591 HCAPLUS
DOCUMENT NUMBER: 127:49213
TITLE: Novel non-pyrogenic bacterial strains and use of the same
INVENTOR(S): Hone, David M.; Powell, Robert J.
PATENT ASSIGNEE(S): University of Maryland At Baltimore, USA; Hone, David M.; Powell, Robert J.
SOURCE: PCT Int. Appl., 124 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9718837	A1	19970529	WO 1996-US19875	19961122
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9722784	A1	19970611	AU 1997-22784	19961122
EP 841941	A1	19980520	EP 1996-945937	19961122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 5997881	A	19991207	US 1997-802371	19970219
PRIORITY APPLN. INFO.:			US 1995-7478	19951122
			WO 1996-US19875	19961122

AB The present invention provides gram-neg. bacterial strains that produce substantially pure non-pyrogenic lipopolysaccharide or lipid A. The present invention also relates to a use of said strains for the prepn. of non-pyrogenic DNA and use of the same for introducing endogenous or foreign genes into animal cells or animal tissue. Further, the present invention relates to a use of said strains for the prepn. of non-pyrogenic bacterial proteins and polysaccharides antigens for use as vaccines.

=> d ind 5

L39 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2002 ACS
IC ICM A61K039-02
ICS A61K048-00; C12P021-06; C12P019-00; C12P019-34; C12P001-04; C12N015-00; C12N001-12; C12N001-20; C07H021-02
CC 15-2 (Immunochemistry)
Section cross-reference(s): 3, 10
ST nonpyrogenic bacteria lipopolysaccharide antigen vaccine DNA
IT Pilins
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(CFA (colonization factor antigen), enterotoxigenic Escherichia coli; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Vaccines
(antigen; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Mutation
(auxotrophic; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Peptides, biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biol. active; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Bird (Aves)
Fish
Mammal (Mammalia)
Reptile
(cell; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Proteins (general), biological studies
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(cochelates; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Virulence (microbial)
(factor; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(htrB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kUsA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdsA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdsB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A

for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(kdtA; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxA**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxB**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(lpxC; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**lpxD**; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Cell cycle
(modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(msbB; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Aeromonas
Animal cells
Animal tissue
Bacteriophage
Bordetella
Brucella
Campylobacter
Chlamydia
Citrobacter
Corynebacterium
Cosmids
Escherichia
Eukaryote (Eukaryotae)
Francisella
Gram-negative bacteria
Haemophilus
Helicobacter
Immunomodulators
Klebsiella
Legionella
Liposomes
Neisseria
Pathogenic microorganism

Phagemids
Plasmids
Pseudomonas
Rhodobacter
Salmonella
Salmonella typhimurium
Shigella
Staphylococcus
Streptococcus
Therapy
Vibrio
Yersinia
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Antisense RNA
Genetic elements
Interferon .alpha.
Interferon .beta.
Interferon .gamma.
Interleukin 1
Interleukin 10
Interleukin 11
Interleukin 12
Interleukin 13
Interleukin 2
Interleukin 3
Interleukin 4
Interleukin 5
Interleukin 6
Interleukin 7
Interleukin 8
Interleukin 9
Monoclonal antibodies
O antigen
Ribozymes
Tumor necrosis factor .alpha.
gp120 (env glycoprotein)
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Calmodulins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Estrogens
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Quaternary ammonium compounds, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Lipid A
Lipopolysaccharides
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

IT Plasmids
(pJGX15C; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Metazoan (Metazoa)
Protozoa
Virus
(pathogen; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Stress (animal)
(response modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Phenotypes
(restriction modification; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Genes (microbial)
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ssc; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Polysaccharides, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(surface; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT DNA
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(topol.; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT Antigens
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(vaccine; non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT 9031-11-2, .beta.-Galactosidase
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)
IT 57-83-0, Progesterone, biological studies 64-20-0D, Tetramethylammonium bromide, tetraacyl derivs. 141-43-5, biological studies 1406-11-7, Polymyxin 2390-68-3 9001-29-0, Factor X 9002-72-6, Growth hormone 9004-10-8, Insulin, biological studies 9035-81-8, Antitrypsin 9061-61-4, Nerve growth factor 11096-26-7, Erythropoietin 20064-29-3D, 1,2-Diacyl deriv. 25104-18-1, Polylysine 62229-50-9, Epidermal growth factor 81627-83-0, M-CSF 83869-56-1, GM-CSF 104162-48-3 168479-03-6
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(non-pyrogenic bacterial strains producing non-pyrogenic lipid A for delivery vaccine genes or DNA into animal cell or tissue)

=> d ibib abs 1

L20 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:622746 HCAPLUS
DOCUMENT NUMBER: 131:334037
TITLE: The active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase. Chemical modification and site-directed mutagenesis
AUTHOR(S): Wyckoff, Timna J. O.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1999), 274(38), 27047-27055
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB UDP-N-acetylglucosamine (UDP-GlcNAc) acyltransferase (**LpxA**) catalyzes the reversible transfer of an R-3-hydroxyacyl chain from R-3-hydroxyacyl-acyl carrier protein to the glucosamine 3-OH of UDP-GlcNAc in the first step of **lipid A** biosynthesis. **Lipid A** is required for the growth and virulence of most **Gram-neg**. bacteria, making its biosynthetic enzymes intriguing targets for the development of new antibacterial agents. **LpxA** is a member of a large family of left-handed .beta.-helical proteins, many of which are acyl- or acetyltransferases. We now demonstrate that histidine-, lysine-, and arginine-specific reagents effectively inhibit **LpxA** of Escherichia coli, whereas serine- and cysteine-specific reagents do not. Using this information in conjunction with multiple sequence alignments, we constructed site-directed alanine substitution mutations of conserved histidine, lysine, and arginine residues. Many of these mutant **LpxA** enzymes show severely decreased specific activities under std. assay conditions. The decrease in activity corresponds to decreased kcat/Km,UDP-GlcNAc values for all the mutants. With the exception of H125A, in which no activity is seen under any assay condition, the decrease in kcat/Km,UDP-GlcNAc mainly reflects an increased Km,UDP-GlcNAc. His125 of E. coli **LpxA** may therefore function as a catalytic residue, possibly as a general base. **LpxA** does not catalyze measurable UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc hydrolysis or UDP-GlcNAc/UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc exchange, arguing against a ping-pong mechanism with an acyl-enzyme intermediate.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ind 1

L20 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2002 ACS
CC 7-5 (Enzymes)
ST Escherichia UDP acetylglucosamine acyltransferase active site
IT Enzyme kinetics
Escherichia coli
(active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)
IT Enzyme functional sites
(active; active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)
IT 105843-69-4
RL: BAC (Biological activity or effector, except adverse); PRP (Properties); BIOL (Biological study)
(**LpxA**; active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)
IT 74-79-3, L-Arginine, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(functional role of residue Arg204; active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)
IT 56-87-1, L-Lysine, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(functional role of residue Lys76; active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)
IT 71-00-1, L-Histidine, biological studies
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)
(functional role of residues His122, His125, His144, and His160; active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase)

=> d ibib abs 2

L20 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:369434 HCAPLUS
DOCUMENT NUMBER: 131:141836
TITLE: **Outer membrane** permeability barrier in Escherichia coli mutants that are defective in the late acyltransferases of **lipid A** biosynthesis
AUTHOR(S): Vaara, Martti; Nurminen, Marjatta
CORPORATE SOURCE: Division of Bacteriology and Immunology, Helsinki University Central Hospital, Helsinki, Finland
SOURCE: Antimicrob. Agents Chemother. (1999), 43(6), 1459-1462
CODEN: AMACCQ; ISSN: 0066-4804
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The tight packing of six fatty acids in the **lipid A** constituent of **lipopolysaccharide** (**LPS**) has been proposed to contribute to the unusually low permeability of the **outer membrane** of **gram-neg.** enteric bacteria to hydrophobic antibiotics. Here it is shown that the Escherichia coli msbB mutant, which elaborates defective, penta-acylated **lipid A**, is practically as resistant to a representative set of hydrophobic solutes (rifampin, fusidic acid, erythromycin, clindamycin, and azithromycin) as the parent-type control strain. The susceptibility index, i.e., the approx. ratio between the MIC for the msbB mutant and that for the parent-type control, was maximally 2.7-fold. In comparison, the rfa mutant defective in the deep core oligosaccharide part of **LPS** displayed indexes ranging from 20 to 64. The **lpxA** and **lpxD lipid A** mutants had indexes higher than 512. Furthermore, the msbB mutant was resistant to glycopeptides (vancomycin, teicoplanin), whereas the rfa, **lpxA**, and **lpxD** mutants were susceptible. The msbB htrB double mutant, which elaborates even-more-defective, partially tetra-acylated **lipid A**, was still less susceptible than the rfa mutant. These findings indicate that hexa-acylated **lipid A** is not a prerequisite for the normal function of the **outer membrane** permeability barrier.
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs 3

L20 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:143286 HCAPLUS
DOCUMENT NUMBER: 118:143286
TITLE: **Outer membrane** permeability barrier to azithromycin, clarithromycin, and roxithromycin in **gram-negative** enteric bacteria
AUTHOR(S): Vaara, Martti
CORPORATE SOURCE: Dep. Bacteriol. Immunol., Univ. Helsinki, Helsinki, 00290, Finland
SOURCE: Antimicrob. Agents Chemother. (1993), 37(2), 354-6
CODEN: AMACCQ; ISSN: 0066-4804
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mutations which severely affect the function of the **outer membrane** of Escherichia coli and Salmonella typhimurium (**lpxA** and firA mutations of **lipid A** synthesis and rfaE mutation of the **lipopolysaccharide** inner-core synthesis) decreased the MICs of erythromycin, roxithromycin, clarithromycin, and azithromycin by factors of 32-512, 32-1024, 64-512, and 16-64, resp. The sensitization factors for 3 other hydrophobic antibiotics (rifampin, fusidic acid, and mupirocin) ranged from 16 to 300. The **outer membrane** permeability-increasing agents polymyxin B nonapeptide (3 .mu.g/mL) and deacylpolymyxin B (1 .mu.g/mL) sensitized wild-type E. coli to azithromycin by factors of 10 and 30, resp. Quant. very similar sensitization to the other macrolides took place. Polymyxin-resistant pmrA mutants of S. typhimurium displayed no cross-resistance to azithromycin. Proteus mirabilis mutants which were sensitized to polymyxin by a factor of .gtoreq.300 to .gtoreq.1,000 had a max. 2-4-fold increase in sensitivity to azithromycin. These results indicate that azithromycin and the other new macrolides use the hydrophobic pathway across the **outer membrane** and that the intact **outer membrane** is an effective barrier against them. The results also indicate that azithromycin, in contrast to polymyxin, does not effectively diffuse through the **outer membrane** by interacting electrostatically with the **lipopolysaccharide**.

=> d ind 3

L20 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2002 ACS
CC 10-5 (Microbial, Algal, and Fungal Biochemistry)
ST **outer membrane** permeability azithromycin clarithromycin roxithromycin; macrolide **outer membrane** permeability enteric bacteria
IT Escherichia coli
Proteus mirabilis
Salmonella typhimurium
(azithromycin and clarithromycin and roxithromycin permeation of **outer membrane** of)
IT Bacteria
(**gram-neg.**, macrolide antibiotics permeation of **outer membrane** of)
IT Antibiotics
(macrolide, **outer membrane** of **gram-neg.** enteric bacteria permeability to)
IT Cell wall
(**outer membrane**, permeability of, to azithromycin and clarithromycin and roxithromycin in enteric bacteria)
IT 114-07-8, Erythromycin 80214-83-1, Roxithromycin 81103-11-9, Clarithromycin 83905-01-5, Azithromycin
RL: BIOL (Biological study)
(**outer membrane** of **gram-neg.** enteric bacteria permeability to)

=> d ibib abs 4

L20 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:213719 HCAPLUS
DOCUMENT NUMBER: 112:213719
TITLE: A mutant of Escherichia coli defective in the first step of endotoxin biosynthesis
AUTHOR(S): Galloway, Susan M.; Raetz, Christian R. H.
CORPORATE SOURCE: Dep. Biochem., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Biol. Chem. (1990), 265(11), 6394-402
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Using localized mutagenesis of whole cells, a temp.-sensitive UDP-N-acetylglucosamine acyltransferase (I) mutant of E. coli that loses all detectable I activity and quickly dies after a shift from 30 to 42.degree. was isolated. I activity and temp. resistance are restored by transforming the mutant with a hybrid plasmid contg. the E. coli gene for I (**lpxA**). In addn., a new assay was developed for quantitating the amt. of **lipid A** (the active component of endotoxin) in E. coli and related **Gram-neg**. strains. Cells are labeled with 32Pi and extd. with CHCl3/MeOH/H2O to remove glycerophospholipids. The residue is then hydrolyzed with 0.2M HCl to liberate the monophosphoryl **lipid A** degrdn. products, each of which bears a single phosphate residue at position 4'. The amt. of **lipid A** is normalized to the total amt. of labeled glycerophospholipid present in the cells. The steady state ratio of **lipid A** to glycerophospholipid in wild-type cells is approx. 0.12. The **lipid A** content of the I mutant is reduced 2-3-fold, and the rate of **lipid A** synthesis is reduced 10-fold when compared to wild-type after 60 min at 42.degree.. These results provide physiol. evidence that I is the major committed step for **lipid A** biosynthesis in E. coli and that **lipid A** is an essential mol.

=> d ind 4

L20 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2002 ACS
CC 10-2 (Microbial Biochemistry)
ST **lipid A** bacteria; UDP acetyglucosamine acyltransferase Escherichia endotoxin
IT Escherichia coli
(endotoxin formation-defective UDP-acetylglucosamine acyltransferase mutant of)
IT Toxins
RL: FORM (Formation, nonpreparative)
(endo-, formation of, Escherichia coli defective in)
IT Glycophospholipids
RL: PROC (Process)
(**lipid A**, isolation of, from Escherichia coli)
IT 105843-69-4, UDP-N-acetylglucosamine acyltransferase
RL: BIOL (Biological study)
(of Escherichia coli, in endotoxin formation)

=> d ibib abs hitstr 1-27

L38 ANSWER 1 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:74079 HCAPLUS
TITLE: Complement activation induced by purified neisseria meningitidis **lipopolysaccharide** (**LPS**), **outer membrane** vesicles, whole bacteria, and an **LPS**-free mutant
AUTHOR(S): Bjerre, Anna; Brusletto, Berit; Mollnes, Tom Eirik; Fritzsonn, Elisabeth; Rosenqvist, Einar; Wedege, Elisabeth; Namork, Ellen; Kierulf, Peter; Brandtzaeg, Petter
CORPORATE SOURCE: Department of Pediatrics, Ulleval University Hospital, Oslo, 0407, Norway
SOURCE: Journal of Infectious Diseases (2002), 185(2), 220-228
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Complement activation is closely assocd. with plasma endotoxin levels in patients with meningococcal infections. This study assessed complement activation induced by purified Neisseria meningitidis **lipopolysaccharide** (Nm-**LPS**), native **outer membrane** vesicles (nOMVs), **LPS**-depleted **outer membrane** vesicles (dOMVs), wild-type meningococci, and an **LPS**-free mutant (**lpxA**-) from the same strain (44/76) in whole blood anticoagulated with the recombinant hirudin analog. Complement activation products (C1rs-C1 inhibitor complexes, C4d, C3bBbP, and terminal SC5b-9 complex) were measured by double-antibody EIAs. Nm-**LPS** was a weak complement activator. Complement activation increased with prepns. contg. nOMVs, dOMVs, and wild-type bacteria at const. **LPS** concns. With the same protein concn., complement activation induced by nOMVs, dOMVs, and the **LPS**-free mutant was equal. The massive complement activation obsd. in patients with fulminant meningococcal septicemia is, presumably, an indirect effect of the massive endotoxemia. **Outer membrane** proteins may be more potent complement activators than meningococcal LPSs.

L38 ANSWER 2 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:9561 HCAPLUS
TITLE: **Outer membrane** composition of a **lipopolysaccharide**-deficient Neisseria meningitidis mutant
AUTHOR(S): Steeghs, Liana; De Cock, Hans; Evers, Evert; Zomer, Bert; Tommassen, Jan; Van der Ley, Peter
CORPORATE SOURCE: Laboratory of Vaccine Research, National Institute of Public Health and the Environment, RIVM, Bilthoven, 3720 BA, Neth.
SOURCE: EMBO Journal (2001), 20(24), 6937-6945
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In the pathogen Neisseria meningitidis, a completely **lipopolysaccharide** (**LPS**)-deficient but viable mutant can be obtained by insertional inactivation of the **lpxA** gene, encoding UDP-GlcNAc acyltransferase required for the first step of **lipid A** biosynthesis. To study how **outer membrane** structure and biogenesis are affected by the absence of this normally major component, inner and **outer membranes**

were sepd. and their compn. analyzed. The expression and assembly of integral **outer membrane** proteins appeared largely unaffected. However, the expression of iron limitation-inducible, cell surface-exposed lipoproteins was greatly reduced. Major changes were seen in the phospholipid compn., with a shift towards phosphatidylethanolamine and phosphatidylglycerol species contg. mostly shorter chain, satd. fatty acids, one of which was unique to the **LPS**-deficient **outer membrane**. The presence of the capsular polysaccharide turned out to be essential for viability without **LPS**, as demonstrated by using a strain in which **LPS** biosynthesis could be switched on or off through a tac promoter-controlled **lpxA** gene. Taken together, these results can help to explain why meningococci have the unique ability to survive without **LPS**.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 3 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:473695 HCAPLUS
DOCUMENT NUMBER: 135:209698
TITLE: Dendritic cell activation and cytokine production induced by group B Neisseria meningitidis: interleukin-12 production depends on **lipopolysaccharide** expression in intact bacteria
AUTHOR(S): Dixon, Garth L. J.; Newton, Phillippa J.; Chain, Benjamin M.; Katz, David; Andersen, Svein Rune; Wong, Simon; Van der Ley, Peter; Klein, Nigel; Callard, Robin E.
CORPORATE SOURCE: Immunobiology Unit, Institute of Child Health, London, WC1N 1EH, UK
SOURCE: Infect. Immun. (2001), 69(7), 4351-4357
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Interactions between dendritic cells (DCs) and microbial pathogens are fundamental to the generation of innate and adaptive immune responses. Upon stimulation with bacteria or bacterial components such as **lipopolysaccharide** (**LPS**), immature DCs undergo a maturation process that involves expression of costimulatory mols., HLA mols., and cytokines and chemokines, thus providing crit. signals for lymphocyte development and differentiation. In this study, we investigated the response of in vitro-generated human DCs to a serogroup B strain of Neisseria meningitidis compared to an isogenic mutant **lpxA** strain totally deficient in **LPS** and purified **LPS** from the same strain. We show that the parent strain, **lpxA** mutant, and meningococcal **LPS** all induce DC maturation as measured by increased surface expression of costimulatory mols. and HLA class I and II mols. Both the parent and **lpxA** strains induced prodn. of tumor necrosis factor alpha (TNF-.alpha.), interleukin-1.alpha. (IL-1.alpha.), and IL-6 in DCs, although the parent was the more potent stimulus. In contrast, high-level IL-12 prodn. was only seen with the parent strain. Compared to intact bacteria, purified **LPS** was a very poor inducer of IL-1.alpha., IL-6, and TNF-.alpha. prodn. and induced no detectable IL-12. Addn. of exogenous **LPS** to the **lpxA** strain only partially restored cytokine prodn. and did not restore IL-12 prodn. These data show that non-**LPS** components of N. meningitidis induce DC maturation, but that **LPS** in the context of the intact bacterium is required for high-level cytokine prodn., esp. that of IL-12. These findings may be useful in assessing

components of N. meningitidis as potential vaccine candidates.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 4 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:429862 HCAPLUS
DOCUMENT NUMBER: 135:192052
TITLE: A Chlamydia trachomatis UDP-N-acetylglucosamine acyltransferase selective for myristoyl-acyl carrier protein. Expression in Escherichia coli and formation of hybrid **lipid A** species
AUTHOR(S): Sweet, Charles R.; Lin, Shanhua; Cotter, Robert J.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (2001), 276(22), 19565-19574
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Chlamydia trachomatis **lipid A** is unusual in that it is acylated with myristoyl chains at the glucosamine 3 and 3' positions. We have cloned and expressed the gene encoding UDP-N-acetylglucosamine 3-O-acyltransferase of C. trachomatis (CtlpxA), the first enzyme of **lipid A** biosynthesis. C. trachomatis **LpxA** displays .apprx.20-fold selectivity for myristoyl-ACP over R/S-3-hydroxymyristoyl-ACP under std. assay conditions, consistent with the proposed structure of C. trachomatis **lipid A**. CtLpxA is the first reported UDP-N-acetylglucosamine acyltransferase that prefers a non-hydroxylated acyl-ACP to a hydroxyacyl-ACP. When CtlpxA was expressed in RO138, a temp.-sensitive **lpxA** mutant of Escherichia coli, five new hybrid **lipid A** species were made in vivo after 2 h at 42.degree.C, in place of Escherichia coli **lipid A**. These compds. were purified and analyzed by matrix-assisted laser desorption ionization/time of flight mass spectrometry. In each case, a myristoyl chain replaced one or both of the ester linked 3-hydroxymyristoyl residues of E. coli **lipid A**. With prolonged growth at 42.degree.C, all the ester-linked 3-hydroxymyristoyl residues were replaced with myristate chains. Re-engineering the structure of E. coli **lipid A** should facilitate the microbiol. prodn. of novel agonists or antagonists of the innate immunity receptor TLR-4, with possible uses as adjuvants or anti-inflammatory agents.
REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 5 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:82554 HCAPLUS
DOCUMENT NUMBER: 135:165889
TITLE: A **lipopolysaccharide**-deficient mutant of Neisseria meningitidis elicits attenuated cytokine release by human macrophages and signals via Toll-like receptor (TLR) 2 but not via TLR4/MD2
AUTHOR(S): Pridmore, Alison C.; Wyllie, David H.; Abdillahi, Fatumo; Steeghs, Liana; van der Ley, Peter; Dower, Steven K.; Read, Robert C.
CORPORATE SOURCE: Division of Molecular and Genetic Medicine, Royal Hallamshire Hospital, University of Sheffield, Sheffield, UK

SOURCE: J. Infect. Dis. (2001), 183(1), 89-96
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Meningococcal disease severity correlates with circulating concns. of **lipopolysaccharide** (**LPS**) and proinflammatory cytokines. Disruption of the **lpxA** gene of N. meningitidis generated a viable strain that was deficient of detectable **LPS**. The potency of wild-type N. meningitidis to elicit tumor necrosis factor (TNF)-.alpha. prodn. by human monocyte-derived macrophages was .apprx.10-fold greater than that of the **lpxA** mutant. Killed wild-type N. meningitidis and its sol. products induced interleukin (IL)-8 and TNF-.alpha. secretion by transfected HeLa cells expressing Toll-like receptor (TLR) 4/MD2, but the **lpxA** mutant was inactive via this pathway. In contrast, both strains induced IL-8 promoter activity in TLR2-transfected HeLa cells. Thus, N. meningitidis contains components other than **LPS** that can elicit biol. responses via pathways that are independent of the TLR4/MD2 receptor system, and TLR2 is one of these alternate pathways. These findings have implications for future therapeutic strategies against meningococcal disease on the basis of the blockade of TLRs and the modulation of **LPS** activity.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 6 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:898319 HCAPLUS
DOCUMENT NUMBER: 134:161772
TITLE: Evidence for an accessory protein function for Toll-like receptor 1 in anti-bacterial responses
AUTHOR(S): Wyllie, D. H.; Kiss-Toth, E.; Visintin, A.; Smith, S. C.; Boussouf, S.; Segal, D. M.; Duff, G. W.; Dower, S. K.
CORPORATE SOURCE: Functional Genomics Group, Division of Molecular and Genetic Medicine, University of Sheffield, Sheffield, S10 2JF, UK
SOURCE: J. Immunol. (2000), 165(12), 7125-7132
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Members of the Toll-like receptor (TLR) family are components of the mammalian anti-microbial response, signaling with a domain closely related to that of IL-1 receptors. In this report the expression and function of TLR1, a TLR of unknown function, are examd. TLR1 is expressed by monocytes, as demonstrated using a novel mAb. Monocytes also express TLR2. TLR1 transfection of HeLa cells, which express neither TLR1 nor TLR2, was not sufficient to confer responsiveness to several microbial exts. However, contransfection of TLR1 and TLR2 resulted in enhanced signaling by HeLa cells to sol. factors released from Neisseria meningitidis relative to the response with either TLR alone. This phenomenon was also seen with high concns. of some prepns. of **LPS**. The N. meningitidis factors recognized by TLR1/TLR2 were not released by N. meningitidis mutant in the **LpxA** gene. Although **LpxA** is required for **LPS** biosynthesis, because cooperation between TLR1 and TLR2 was not seen with all **LPS** prepns., the microbial component(s) TLR1/2 recognizes is likely to be a complex of **LPS** and other mols. or a compd. metabolically and chem. related to **LPS**. The functional IL-1R consists of a heterodimer; this report suggests a similar mechanism for TLR1 and TLR2,

for certain agonists. These data further suggest that mammalian responsiveness to some bacterial products may be mediated by combinations of TLRs, suggesting a mechanism for diversifying the repertoire of Toll-mediated responses.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 7 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:675643 HCAPLUS
DOCUMENT NUMBER: 132:31545
TITLE: An unusual arrangement of pur and lpx genes in the photosynthetic purple sulfur bacterium Allochromatium vinosum
AUTHOR(S): Chen, Yie Lane; Dincturk, H. Benan; Knaff, David B.
CORPORATE SOURCE: Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX, 79409-1061, USA
SOURCE: Mol. Biol. Rep. (1999), 26(3), 195-199
CODEN: MLBRBU; ISSN: 0301-4851
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nucleotide sequence of a 1634 bp DNA fragment from the photosynthetic purple sulfur bacterium Allochromatium vinosum contains one complete and two partial open reading frames. Sequence comparisons to genes from other organisms suggest that this A. vinosum DNA fragment contains, starting from the 5' end, the following: (1) 234 bp at the 3' end of the A. vinosum purH gene, coding for 78 amino acids at the C-terminus of the bi-functional 5'-phosphoribosyl-5-aminoimidazole-4-carboxamide formyltransferase/IMP cyclohydrolase (EC 2.1.2.3), an enzyme involved in de novo purine biosynthesis; (2) 777 bp of the A. vinosum **lpxA** gene, coding for all 259 amino acids of the UDP-N-acetylglucosamine-O-acyltransferase, an enzyme involved in **lipid A** biosynthesis; and (3) 567 bp at the 5' end of the A. vinosum purD gene, coding for 189 amino acids at the N-terminus of 5'-phosphoribosyl glycinamide synthetase (EC 6.3.4.13), a second enzyme involved in de novo purine biosynthesis. The presence of a gene coding for an enzyme involved in **lipid A** biosynthesis between two genes coding for enzymes of the de novo purine biosynthesis pathway represents a unique arrangement of these genes.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 8 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:622746 HCAPLUS
DOCUMENT NUMBER: 131:334037
TITLE: The active site of Escherichia coli UDP-N-acetylglucosamine acyltransferase. Chemical modification and site-directed mutagenesis
AUTHOR(S): Wyckoff, Timna J. O.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1999), 274(38), 27047-27055
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB UDP-N-acetylglucosamine (UDP-GlcNAc) acyltransferase (**LpxA**) catalyzes the reversible transfer of an R-3-hydroxyacyl chain from R-3-hydroxyacyl-acyl carrier protein to the glucosamine 3-OH of UDP-GlcNAc

in the first step of **lipid A** biosynthesis. **Lipid A** is required for the growth and virulence of most **Gram-neg**. bacteria, making its biosynthetic enzymes intriguing targets for the development of new antibacterial agents. **LpxA** is a member of a large family of left-handed .beta.-helical proteins, many of which are acyl- or acetyltransferases. We now demonstrate that histidine-, lysine-, and arginine-specific reagents effectively inhibit **LpxA** of Escherichia coli, whereas serine- and cysteine-specific reagents do not. Using this information in conjunction with multiple sequence alignments, we constructed site-directed alanine substitution mutations of conserved histidine, lysine, and arginine residues. Many of these mutant **LpxA** enzymes show severely decreased specific activities under std. assay conditions. The decrease in activity corresponds to decreased kcat/Km,UDP-GlcNAc values for all the mutants. With the exception of H125A, in which no activity is seen under any assay condition, the decrease in kcat/Km,UDP-GlcNAc mainly reflects an increased Km,UDP-GlcNAc. His125 of E. coli **LpxA** may therefore function as a catalytic residue, possibly as a general base. **LpxA** does not catalyze measurable UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc hydrolysis or UDP-GlcNAc/UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc exchange, arguing against a ping-pong mechanism with an acyl-enzyme intermediate.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 9 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:369434 HCAPLUS
DOCUMENT NUMBER: 131:141836
TITLE: **Outer membrane** permeability barrier in Escherichia coli mutants that are defective in the late acyltransferases of **lipid A** biosynthesis
AUTHOR(S): Vaara, Martti; Nurminen, Marjatta
CORPORATE SOURCE: Division of Bacteriology and Immunology, Helsinki University Central Hospital, Helsinki, Finland
SOURCE: Antimicrob. Agents Chemother. (1999), 43(6), 1459-1462
CODEN: AMACCQ; ISSN: 0066-4804
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The tight packing of six fatty acids in the **lipid A** constituent of **lipopolysaccharide** (**LPS**) has been proposed to contribute to the unusually low permeability of the **outer membrane** of **gram-neg**. enteric bacteria to hydrophobic antibiotics. Here it is shown that the Escherichia coli msbB mutant, which elaborates defective, penta-acylated **lipid A**, is practically as resistant to a representative set of hydrophobic solutes (rifampin, fusidic acid, erythromycin, clindamycin, and azithromycin) as the parent-type control strain. The susceptibility index, i.e., the approx. ratio between the MIC for the msbB mutant and that for the parent-type control, was maximally 2.7-fold. In comparison, the rfa mutant defective in the deep core oligosaccharide part of **LPS** displayed indexes ranging from 20 to 64. The **lpxA** and **lpxD lipid A** mutants had indexes higher than 512. Furthermore, the msbB mutant was resistant to glycopeptides (vancomycin, teicoplanin), whereas the rfa, **lpxA**, and **lpxD** mutants were susceptible. The msbB htrB double mutant, which elaborates even-more-defective, partially tetra-acylated **lipid A**, was still less susceptible than the rfa mutant. These findings indicate that hexa-acylated **lipid A** is

not a prerequisite for the normal function of the **outer membrane** permeability barrier.
REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 10 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:288450 HCAPLUS
DOCUMENT NUMBER: 131:85388
TITLE: **Outer membrane** permeability of the antibiotic-supersusceptible **lipid A** mutants of Escherichia coli to hydrophobic steroid probes
AUTHOR(S): Plesiat, Patrick; Vaara, Martti
CORPORATE SOURCE: Laboratoire de Bacteriologie, Faculte de Medecine, Besancon, Fr.
SOURCE: J. Antimicrob. Chemother. (1999), 43(4), 608-610
CODEN: JACHDX; ISSN: 0305-7453
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It was demonstrated that the diffusion rate of testosterone hemisuccinate through the **lpxA**-type **outer membrane** (OM) is much higher than that through the Re-type OM. P values (nm/s) are given for testosterone and testosterone hemisuccinate in the **lipid A** mutants of E. colit, **lpxA** and **lpxD**, their isogenic parent-type strains, and a mutant strain of E. coli, D21f2, with a defective inner core oligosaccharide.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 11 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:797426 HCAPLUS
DOCUMENT NUMBER: 130:135835
TITLE: Hydrocarbon rulers in UDP-N-acetylglucosamine acyltransferases
AUTHOR(S): Wyckoff, Timna J. O.; Lin, Shanhua; Cotter, Robert J.; Dotson, Garry D.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Biol. Chem. (1998), 273(49), 32369-32372
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB UDP-GlcNAc acyltransferase (**LpxA**), the first enzyme of **lipid A** biosynthesis, catalyzes the transfer of an acyl chain activated on acyl carrier protein (ACP) to UDP-GlcNAc. **LpxAs** are very selective for the lengths of their acyl donor substrates. Escherichia coli **LpxA** prefers R-3-hydroxymyristoyl-ACP to R-3-hydroxydecanoyl-ACP by a factor of .apprx.1000, whereas Pseudomonas aeruginosa **LpxA** prefers the opposite. E. coli G173M **LpxA** and the reciprocal P. aeruginosa M169G **LpxA** show reversed substrate selectivity in vitro and in vivo, demonstrating the existence of precise hydrocarbon rulers in **LpxAs**.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L38 ANSWER 12 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:54284 HCAPLUS

DOCUMENT NUMBER: 128:201541
TITLE: Expression cloning of a Pseudomonas gene encoding a hydroxydecanoyl-acyl carrier protein-dependent UDP-GlcNAc acyltransferase
AUTHOR(S): Dotson, Garry D.; Kaltashov, Igor A.; Cotter, Robert J.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA
SOURCE: J. Bacteriol. (1998), 180(2), 330-337
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB UDP-N-acetylglucosamine-3-O-acyltransferase (UDP-GlcNAc acyltransferase) catalyzes the first step of **lipid A** biosynthesis (M. S. Anderson and C. R. H. Raetz, J. Biol. Chem. 262:5159-5169, 1987). We here report the isolation of the **lpxA** gene of Pseudomonas aeruginosa from a library of Pseudomonas strain PAO1 expressed in Escherichia coli LE392 (J. Lightfoot and J. S. Lam, J. Bacteriol. 173:5624-5630, 1991). Pseudomonas **lpxA** encodes a 10-carbon-specific UDP-GlcNAc acyltransferase, whereas the E. coli transferase is selective for a 14-carbon acyl chain. Recombinant cosmid 1137 enabled prodn. of a 3-hydroxydecanoyl-specific UDP-GlcNAc acyltransferase in E. coli. It was identified by assaying lysozyme-EDTA lysates of individual members of the library with 3-hydroxydecanoyl-acyl carrier protein (ACP) as the substrate. Cosmid 1137 contained a 20-kb insert of P. aeruginosa DNA. The **lpxA** gene region was localized to a 1.3-kb SalI-PstI fragment. Sequencing revealed that it contains one complete open reading frame (777 bp) encoding a new **lpxA** homolog. The predicted Pseudomonas **LpxA** is 258 amino acids long and contains 21 complete hexapeptide repeating units, spaced in approx. the same manner as the 24 repeats of E. coli **LpxA**. The P. aeruginosa UDP-GlcNAc acyltransferase is 54% identical and 67% similar to the E. coli enzyme. A plasmid (pGD3) contg. the 1.3-kb SalI-PstI fragment complemented E. coli RO138, a temp.-sensitive mutant harboring lpxA2. **LpxA** assays of exts. of this construct indicated that it is >1,000-fold more selective for 3-hydroxydecanoyl-ACP than for 3-hydroxymyristoyl-ACP. Mass spectrometry of **lipid A** isolated from this strain by hydrolysis at pH 4.5 revealed [M-H]- 1,684.5 (vs. 1,796.5 for wild-type **lipid A**), consistent with 3-hydroxydecanoate rather than 3-hydroxymyristate at positions 3 and 3'.

L38 ANSWER 13 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:239766 HCAPLUS
DOCUMENT NUMBER: 126:339433
TITLE: Isolation and characterization of the Neisseria meningitidis **lpxD**-fabZ-**lpxA** gene cluster involved in **lipid A** biosynthesis
AUTHOR(S): Steeghs, Liana; Jennings, Michael P.; Poolman, Jan T.; van der Ley, Peter
CORPORATE SOURCE: Laboratory of Vaccine Development and Immune Mechanisms, National Institute of Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, P.O. Box 1, BA Bilthoven, 3720, Neth.
SOURCE: Gene (1997), 190(2), 263-270
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **lpxD**-fabZ-**lpxA** gene cluster involved in **lipid A** biosynthesis in Neisseria meningitidis has been cloned and sequenced. By complementation of a temp.-sensitive E. coli **lpxD** mutant, we first cloned a meningococcal chromosomal fragment that carries the **lpxD** homolog. Cloning and sequence anal. of chromosomal DNA downstream of **lpxD** revealed the presence of the fabZ and **lpxA** genes. This gene cluster shows high homol. to the corresponding genes from several other bacterial species. The **LpxA** and **LpxD** proteins catalyze early steps in the **lipid A** biosynthetic pathway, adding the O- and N-linked 3-OH fatty acyl chains, resp. In E. coli and N. meningitidis, **LpxD** has the same specificity, in both cases adding 3-OH myristoyl chains; in contrast to E. coli, the meningococcal **LpxA** protein is presumed to add 3-OH lauroyl chains instead. The established sequence points the way to further expts. to define the basis for this difference in specificity, and should allow modification of meningococcal **lipid A** biosynthesis through gene exchange.

L38 ANSWER 14 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:692667 HCAPLUS
DOCUMENT NUMBER: 126:57210
TITLE: Association of **lipid A** disaccharide synthase with aerobic glycerol-3-phosphate dehydrogenase in extracts of Escherichia coli
AUTHOR(S): Milla, Marcos E.; Raetz, Christian R. H.
CORPORATE SOURCE: Department of Biochemistry, Duke University Medical Center, Durham, USA
SOURCE: Biochim. Biophys. Acta (1996), 1304(3), 245-253
CODEN: BBACAQ; ISSN: 0006-3002
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Variants of the Escherichia coli UDP-GlcNAc O-acyltransferase (**LpxA**) and of the **lipid A** disaccharide synthase (**LpxB**) contg. affinity chromatog. tags (C-terminal histidine8 [H8] tails) were constructed in order to investigate whether or not these enzymes interact with other E. coli proteins. These variants (**LpxA**-H8 and **LpxB**-H8) had specific activities in vitro that were similar to wild-type enzymes. Crude exts. made from E. coli cells expressing **LpxA**-H8 or **LpxB**-H8 were chromatographed over Ni2+-NTA-Agarose, and proteins purifying with the tagged proteins were identified by SDS-PAGE, followed by blotting and N-terminal microsequencing. At high levels of **LpxB**-H8 expression, two heat-shock proteins (DnaK and GroEL) were assocd. with the disaccharide synthase, but not with the acyltransferase. Another major protein recovered with **LpxB**-H8 (both at low and high levels of expression) was the aerobic glycerol-3-phosphate dehydrogenase (GlpD). The latter interaction was specific, since GlpD did not bind the affinity resin when the affinity tag was present on the UDP-GlcNAc O-acyltransferase (**LpxA**-H8). Velocity centrifugation expts. indicated that both wild-type **LpxB** and GlpD sedimented together under some conditions, but these aggregates were smaller than and distinct from inner membranes. These findings suggest a possible new mechanism by which the biosynthetic pathways for **lipid A** and glycerophospholipids may be coordinated.

L38 ANSWER 15 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:633254 HCAPLUS
DOCUMENT NUMBER: 126:1992

TITLE: Cloning and expression of genes encoding **lipid A** biosynthesis from Haemophilus influenzae type b
AUTHOR(S): Servos, Spiros; Khan, Shahid; Maskell, Duncan
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science Technology, Medicine, Exhibition Road, London, SW7 2AY, UK
SOURCE: Gene (1996), 175(1/2), 137-141
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Genes similar to Escherichia coli lpx genes (encoding enzymes required for the biosynthesis of **lipid A**) have been cloned from Haemophilus influenzae type b using a hybridization-based strategy. The derived amino acid sequences are highly homologous to their E. coli counterparts. The genes appear in the same order in both E. coli and H. influenzae, but the intergenic regions differ. H. influenzae **lpxA** and **lpxB** have been expressed in E. coli minicells and they encode proteins of the predicted sizes. Both H. influenzae **lpxA** and **lpxB** are able to complement temp.-sensitive mutants in the equiv. genes in E. coli. This provides evidence that the genetic manipulation of lpx genes to generate altered **lipid A** mols. may be possible.

L38 ANSWER 16 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:988997 HCAPLUS
DOCUMENT NUMBER: 124:50366
TITLE: Comparison of the phenotypes of the **lpxA** and **lpxD** mutants of Escherichia coli
AUTHOR(S): Vuorio, Riitta; Vaara, Martti
CORPORATE SOURCE: Department of Bacteriology and Immunology, PO Box 21 (Haartmaninkatu 3), University of Helsinki, Helsinki, SF-00014, Finland
SOURCE: FEMS Microbiol. Lett. (1995), 134(2-3), 227-32
CODEN: FMLED7; ISSN: 0378-1097
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors compared the phenotype of two thermosensitive Escherichia coli mutants defective in **lipid A** biosynthesis, i.e. SM101 (**lpxA**) and CDH23-213 (**lpxD**). More than 40% of the periplasmic 27-kDa marker enzyme .beta.-lactamase was released from SM101 at 28.degree.. At this temp., the mutant still grew with a generation time (67 min), not much longer than that of the parent control strain (57 min). CDH23-213 released .beta.-lactamase only at higher temps. SM101 and CDH23-213 were both unable to grow in hypo-osmotic conditions. Derivs. of SM101 and CDH23-213 with mdoA--Tn10 had identical phenotypes (including thermosensitivity and defective **outer membrane** permeability barrier to hydrophobic probes) to those of SM101 and CDH23-213, indicating that the potential loss of membrane-derived oligosaccharides (MDO) did not explain these phenotypic properties. A method for the estn. of **lipid A** synthesis rate was developed.

L38 ANSWER 17 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:937691 HCAPLUS
DOCUMENT NUMBER: 124:49323
TITLE: A left-handed parallel .beta. helix in the structure of UDP-N-acetylglucosamine acyltransferase
AUTHOR(S): Raetz, Christian R. H.; Roderick, Steven L.

CORPORATE SOURCE: Department Biochemistry, Duke University Medical Center, Durham, NC, 22710, USA
SOURCE: Science (Washington, D. C.) (1995), 270(5238), 997-1000
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB UDP-N-acetylglucosamine 3-O-acyltransferase (**LpxA**) catalyzes the transfer of (R)-3-hydroxymyristic acid from its acyl carrier protein thioester to UDP-N-acetylglucosamine. **LpxA** is the first enzyme in the **lipid A** biosynthetic pathway and is a target for the design of antibiotics. The x-ray crystal structure of **LpxA** has been detd. to 2.6 angstrom resoln. and reveals a domain motif composed of parallel .beta. strands, termed a left-handed parallel .beta. helix (L.beta.H). This unusual fold displays repeated violations of the protein folding constraint requiring right-handed crossover connections between strands of parallel .beta. sheets and may be present in other enzymes that share amino acid sequence homol. to the repeated hexapeptide motif of **LpxA**.

L38 ANSWER 18 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:428163 HCAPLUS
DOCUMENT NUMBER: 121:28163
TITLE: Characterization of a Rickettsia rickettsii DNA fragment analogous to the fir A-ORF17-**lpxA** region of Escherichia coli
AUTHOR(S): Shaw, Edward I.; Wood, David O.
CORPORATE SOURCE: Dep. Microbiol. Immunol., Univ. South Alabama, Mobile, AL, 36688, USA
SOURCE: Gene (1994), 140(1), 109-13
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A firA and **lpxA** genes, as well as an ORF coding for a putative 16-kDa protein of unknown function, have been identified and characterized in the obligate intracellular bacterium, Rickettsia rickettsii. This is the first description of these genes, which code for enzymes involved in the biosynthesis of **lipid A**, in a species outside of the Enterobacteriaceae. The deduced amino acid (aa) sequences of FirA, ORF16 and **LpxA** of R. rickettsii, when compared to their Escherichia coli analogs, exhibited 35, 44 and 41% aa identity, resp. In addn., the order of genes in R. rickettsii, firA-ORF16-**lpxA**, was identical to that found in E. coli; however, the spacing between the rickettsial genes was greater. Interestingly, the R. rickettsii FirA and **LpxA** deduced proteins retain an unusual hexapeptide repeat pattern found in E. coli and Salmonella typhimurium FirA/Ssc and E. coli **LpxA**, as well as other acyltransferases, providing addnl. support for the importance of this structure.

L38 ANSWER 19 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:239054 HCAPLUS
DOCUMENT NUMBER: 120:239054
TITLE: The novel hexapeptide motif found in the acyltransferases **LpxA** and **LpxD** of **lipid A** biosynthesis is conserved in various bacteria
AUTHOR(S): Vuorio, Riitta; Harkonen, Taina; Tolvanen, Martti; Vaara, Martti
CORPORATE SOURCE: Univ. Helsinki, Helsinki, Finland
SOURCE: FEBS Lett. (1994), 337(3), 289-92

CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two bacterial acyltransferases (**LpxA** of Escherichia coli, **LpxD** of E. coli and Salmonella typhimurium) have previously been shown to consist of a very unusual tandem-repeat structure with tens of repeating hexapeptides (24 hexapeptides in **LpxA**, 26 in **LpxD**). By sequencing **LpxD** of Yersinia enterocolitica (a distant relative of E. coli and S. typhimurium within Enterobacteriaceae) as well as **LpxA** of S. typhimurium and Y. enterocolitica, and by analyzing the existing data on these enzymes of Rickettsia rickettsia, it was now shown that the hexapeptide repeat pattern is a very conservative property of these enzymes. Even though the overall homol. (allowing equiv. amino acids) between the four proteins was only 59% in **LpxA** and 58% in **LpxD**, the homol. in the first residue of each hexapeptide was 87% in **LpxA** and 100% in **LpxD**. Secondary structure prediction by Predict Protein server suggested a very strong beta strand dominance in all the hexad regions. Accordingly, **LpxA** and **LpxD** of various bacterial origins can now be regarded as structurally very unusual enzymes, largely consisting of hexad repeats.

L38 ANSWER 20 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:599406 HCAPLUS
DOCUMENT NUMBER: 119:199406
TITLE: The firA gene of Escherichia coli encodes UDP-3-O-(R-3-hydroxymyristoyl)-glucosamine N-acyltransferase. The third step of endotoxin biosynthesis
AUTHOR(S): Kelly, Theresa M.; Stachula, Sheryl A.; Raetz, Christian R. H.; Anderson, Matt S.
CORPORATE SOURCE: Dep. Biochem., Merck Res. Lab., Rahway, NJ, 07065, USA
SOURCE: J. Biol. Chem. (1993), 268(26), 19866-74
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The possibility that the firA gene of Escherichia coli (Dicker, I. B.; Seetharam, S., 1991) might function in **lipid A** biosynthesis was examd. based on its homol. to the **lpxA** gene, which encodes UDP-N-acetylglucosamine O-acyl-transferase, the first enzyme in **lipid A** formation. Exts. of a temp.-sensitive firA mutant, RL-25, were assayed for their ability to acylate UDP-GlcNAc, using a coupled assay. The results suggested that exts. of RL-25 might be defective in the third enzyme of this pathway, the UDP-3-O-(R-3-hydroxymyristoyl)-glucosamine N-acyltransferase. Living cells of RL-25 also displayed a 5-fold decreased rate of **lipid A** biosynthesis at the nonpermissive temp. as judged by a 32Pi incorporation assay. In order to examine N-acyltransferase activity directly, the substrate [.alpha.-32P]UDP-3-O-(R-3-hydroxymyristoyl)-GlcN was synthesized enzymically. N-Acyltransferase specific activity in RL-25 exts. was reduced to less than 10% of wild-type. When the wild-type firA gene was cloned into a T7-based expression vector, N-acyltransferase specific activity increased almost 360-fold relative to wild-type exts., demonstrating that firA is the structural gene for the enzyme. The N-acyltransferase displays abs. specificity for the R-3-OH moiety of R-3-hydroxymyristoyl-ACP, as does the O-acetyltransferase, consistent with the placement fo R-3-hydroxymyristate in E. coli **lipid A**
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L38 ANSWER 21 OF 27 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:143286 HCAPLUS
DOCUMENT NUMBER: 118:143286
TITLE: **Outer membrane** permeability barrier to azithromycin, clarithromycin, and roxithromycin in **gram-negative** enteric bacteria
AUTHOR(S): Vaara, Martti
CORPORATE SOURCE: Dep. Bacteriol. Immunol., Univ. Helsinki, Helsinki, 00290, Finland
SOURCE: Antimicrob. Agents Chemother. (1993), 37(2), 354-6
CODEN: AMACCQ; ISSN: 0066-4804
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mutations which severely affect the function of the **outer membrane** of Escherichia coli and Salmonella typhimurium (**lpxA** and firA mutations of **lipid A** synthesis and rfaE mutation of the **lipopolysaccharide** inner-core synthesis) decreased the MICs of erythromycin, roxithromycin, clarithromycin, and azithromycin by factors of 32-512, 32-1024, 64-512, and 16-64, resp. The sensitization factors for 3 other hydrophobic antibiotics (rifampin, fusidic acid, and mupirocin) ranged from 16 to 300. The **outer membrane** permeability-increasing agents polymyxin B nonapeptide (3 .mu.g/mL) and deacylpolymyxin B (1 .mu.g/mL) sensitized wild-type E. coli to azithromycin by factors of 10 and 30, resp. Quant. very similar sensitization to the other macrolides took place. Polymyxin-resistant pmrA mutants of S. typhimurium displayed no cross-resistance to azithromycin. Proteus mirabilis mutants which were sensitized to polymyxin by a factor of .gtoreq.300 to .gtoreq.1,000 had a max. 2-4-fold increase in sensitivity to azithromycin. These results indicate that azithromycin and the other new macrolides use the hydrophobic pathway across the **outer membrane** and that the intact **outer membrane** is an effective barrier against them. The results also indicate that azithromycin, in contrast to polymyxin, does not effectively diffuse through the **outer membrane** by interacting electrostatically with the **lipopolysaccharide**.

L38 ANSWER 22 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:96464 HCAPLUS
DOCUMENT NUMBER: 118:96464
TITLE: The Ssc protein of enteric bacteria has significant homology to the acyltransferase **Lpxa** of **lipid A** biosynthesis, and to three acetyltransferases
AUTHOR(S): Vuorio, Riitta; Hirvas, Laura; Vaara, Martti
CORPORATE SOURCE: Dep. Bacteriol. Immunol., Univ. Helsinki, Helsinki, 00290, Finland
SOURCE: FEBS Lett. (1991), 292(1-2), 90-4
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Ssc protein, a novel essential protein affecting the function of the enterobacterial **outer membrane**, best matched with **LpxA** (UDP-N-acetylglucosamine 3-hydroxymyristoyl transferase, which catalyzes the first step of **lipid A** biosynthesis) in a protein homol. search. The corresponding genes, located 0.56 kb apart, were 46.7% identical. The search also revealed homol. to the bacterial acetyltransferases LacA and NodL, as well as to a hypothetical protein Yglm. Residues 109-149 of Ssc displayed the highest homol. with these proteins, and was also homologous with another bacterial

acetyltransferase, CysE, and three other bacterial proteins, two of which are hypothetical. This region and the corresponding regions of all other proteins were found to have a peculiar repeated hexapeptide pattern. Each hexapeptide unit starts with isoleucine, leucine, or valine. In most units, the second residue is glycine and the fifth residue either valine or alanine.

L38 ANSWER 23 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1990:213719 HCAPLUS
DOCUMENT NUMBER: 112:213719
TITLE: A mutant of Escherichia coli defective in the first step of endotoxin biosynthesis
AUTHOR(S): Galloway, Susan M.; Raetz, Christian R. H.
CORPORATE SOURCE: Dep. Biochem., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Biol. Chem. (1990), 265(11), 6394-402
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Using localized mutagenesis of whole cells, a temp.-sensitive UDP-N-acetylglucosamine acyltransferase (I) mutant of E. coli that loses all detectable I activity and quickly dies after a shift from 30 to 42.degree. was isolated. I activity and temp. resistance are restored by transforming the mutant with a hybrid plasmid contg. the E. coli gene for I (**lpxA**). In addn., a new assay was developed for quantitating the amt. of **lipid A** (the active component of endotoxin) in E. coli and related **Gram-neg**. strains. Cells are labeled with 32Pi and extd. with CHCl3/MeOH/H2O to remove glycerophospholipids. The residue is then hydrolyzed with 0.2M HCl to liberate the monophosphoryl **lipid A** degrdn. products, each of which bears a single phosphate residue at position 4'. The amt. of **lipid A** is normalized to the total amt. of labeled glycerophospholipid present in the cells. The steady state ratio of **lipid A** to glycerophospholipid in wild-type cells is approx. 0.12. The **lipid A** content of the I mutant is reduced 2-3-fold, and the rate of **lipid A** synthesis is reduced 10-fold when compared to wild-type after 60 min at 42.degree.. These results provide physiol. evidence that I is the major committed step for **lipid A** biosynthesis in E. coli and that **lipid A** is an essential mol.

L38 ANSWER 24 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:70108 HCAPLUS
DOCUMENT NUMBER: 110:70108
TITLE: First committed step of **lipid A** biosynthesis in Escherichia coli: sequence of the **lpxA** gene
AUTHOR(S): Coleman, Jack; Raetz, Christian R. H.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Bacteriol. (1988), 170(3), 1268-74
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The min 4 region of the E. coli genome contains genes (**lpxA** and **lpxB**) that encode proteins involved in **lipid A** biosynthesis. The sequence of 1,350 base pairs of DNA upstream of the **lpxB** gene was detd. This fragment of DNA contains the complete coding sequence for the 28.0-kilodalton **lpxA** gene product and an upstream open reading frame capable of encoding a 17-kilodalton protein

(ORF17). There appears to be an addnl. open reading frame (ORF2) immediately upstream of ORF17. The initiation codon for **lpxA** is a GUG codon, and the start codon for ORF17 is apparently a UUG codon. The start and stop codons overlap between ORF2 and ORF17, ORF17 and **lpxA**, and **lpxA** and **lpxB**. This overlap is suggestive of translational coupling and argues that the genes are cotranscribed.

L38 ANSWER 25 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:125388 HCAPLUS
DOCUMENT NUMBER: 108:125388
TITLE: Sequence analysis of the Escherichia coli dnaE gene
AUTHOR(S): Tomasiewicz, Henry G.; McHenry, Charles S.
CORPORATE SOURCE: Health Sci. Cent., Univ. Colorado, Denver, CO, 80262, USA
SOURCE: J. Bacteriol. (1987), 169(12), 5735-44
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The sequence of a 4350-nucleotide region of the E. coli chromosome that contains dnaE, the structural gene for the .alpha. subunit of DNA polymerase III holoenzyme was detd. The dnaE gene appeared to be part of an operon contg. .gtoreq.3 other genes: 5'-**lpxB** -ORF23-dnaE-ORF37-3' (ORF= open reading frame). The **lpxB** gene encodes **lipid A** disaccharide synthase, an enzyme essential for cell growth and division. The termination codons of **lpxB** and ORF23 overlapped the initiation codons of ORF23 and dnaE, resp., suggesting translational coupling. No rho-independent transcription termination sequences were obsd. A potential internal transcriptional promoter was found preceding dnaE. Deletion of the -35 region of this promoter abolished dnaE expression in plasmids lacking addnl. upstream sequences. From the deduced amino acid sequence, .alpha. had a mol. wt. of 129,920 and an isoelec. point of 4.93 for the denatured protein. ORF23 encoded a more basic protein (pI 7.11) with a mol. wt. of 23,228.

L38 ANSWER 26 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:125387 HCAPLUS
DOCUMENT NUMBER: 108:125387
TITLE: Nucleotide sequence of the Escherichia coli gene for **lipid A** disaccharide synthase
AUTHOR(S): Crowell, Dring N.; Reznikoff, William S.; Raetz, Christian R. H.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin-Madison, Madison, WI, 53706, USA
SOURCE: J. Bacteriol. (1987), 169(12), 5727-34
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **lpxB** gene of E. coli, believed to be the structural gene for **lipid A** disaccharide synthase, is located in the min 4 region of the chromosome. It is adjacent to and clockwise of the **lpxA** gene, which is thought to encode UDP-N-acetylglucosamine acyltransferase. Preliminary evidence suggests that **lpxA** and **lpxB** are cotranscribed in the clockwise direction and thus constitute part of a previously unknown operon. The complete nucleotide sequence of a 1522-base-pair PvuII-HincII fragment known to carry the **lpxB** gene is reported. This sequence contained an open reading frame of 1149 base pairs, in agreement with the predicted size, location, and orientation of **lpxB**. There was a second open reading frame

5' to, and in the same orientation as, **lpxB** that corresponded to **lpxA**. The ochre codon terminating **lpxA** overlapped the methionine codon identified as the initiation codon for **lpxB**, suggesting that these genes are cotranscribed and translationally coupled. A third open reading frame began at the 3' end of **lpxB** with analogous overlap between the opal codon terminating **lpxB** and the methionine codon that putatively initiates translation downstream of **lpxB** in the clockwise direction. Apparently, .gtoreq.3 genes constitute a translationally coupled operon in the min 4 region of the E. coli chromosome.

L38 ANSWER 27 OF 27 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1987:14156 HCAPLUS
DOCUMENT NUMBER: 106:14156
TITLE: Molecular cloning of the genes for **lipid A** disaccharide synthase and UDP-N-acetylglucosamine acyltransferase in Escherichia coli
AUTHOR(S): Crowell, Dring N.; Anderson, Matt S.; Raetz, Christian R. H.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: J. Bacteriol. (1986), 168(1), 152-9
CODEN: JOBAAY; ISSN: 0021-9193
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Several enzymes have been discovered recently in crude exts. of E. coli that appear to be involved in the biosynthesis of the **lipid A** component of **lipopolysaccharide**. Two of these are **lipid A** disaccharide synthase [105843-81-0] and UDP-N-acetylglucosamine acyltransferase [105843-69-4]. **Lipid A** disaccharide synthase activity is barely detectable in cells harboring a lesion in the **lpxB** (pgsB) gene. The **lpxB** gene was subcloned from plasmid pLC26-43 of the Clarke and Carbon collection (L. Clarke and J. Carbon, 1976) and localized it to a 1.7-kilobase-pair fragment of DNA counterclockwise of dnaE on the E. coli chromosome. A new gene (**lpxA**) was located adjacent to and counterclockwise of **lpxB** that encodes or controls UDP-N-acetylglucosamine acyltransferase. Apparently, **lpxB** and **lpxA** are transcribed in the clockwise direction and they may be cotranscribed.